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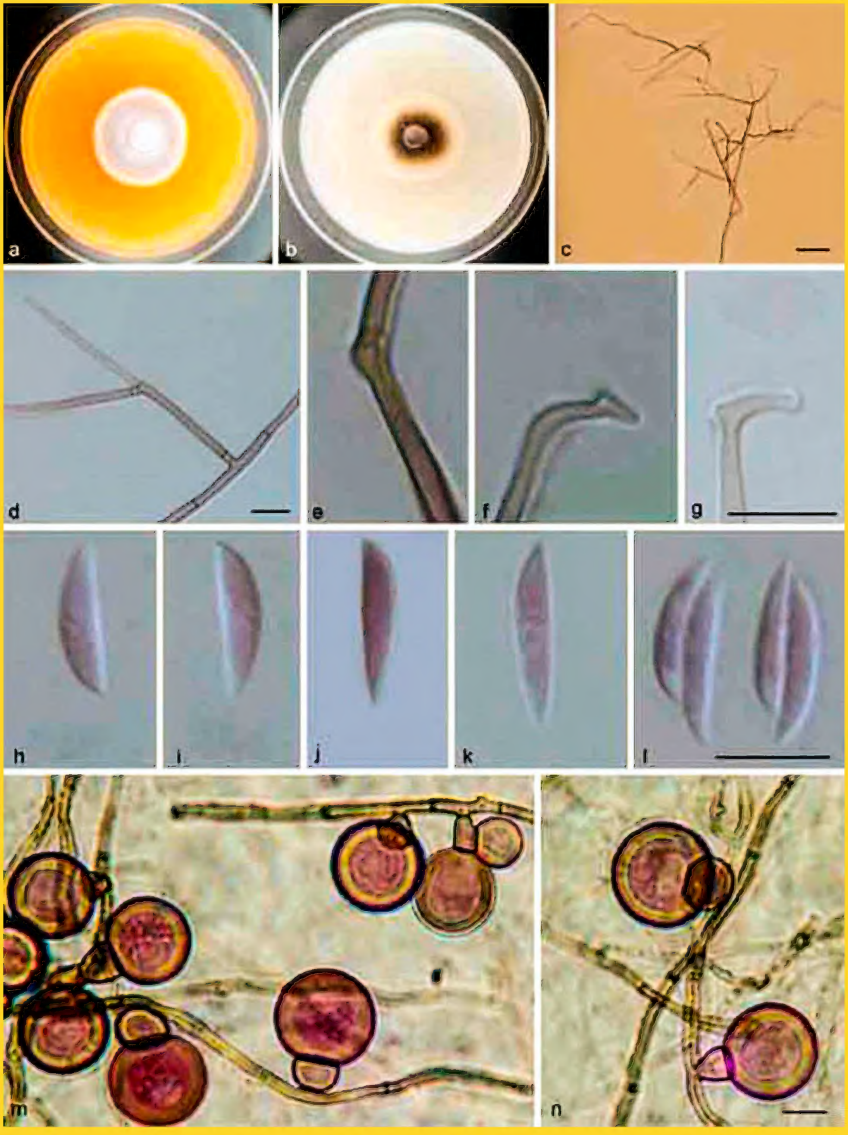

Guayaquilia cubensis sp. nov.
(Magdama & al. — FIG. 3, p. 509)

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MYCOTAXON

VOLUME ONE HUNDRED THIRTY-FIVE (3) — TABLE OF CONTENTS

Nomenclatural novelties & typifications vii
Reviewers ix
Corrigenda x
From the Editor xi
2020 submission procedure xiii

Taxonomy

Lepiota punaensis sp. nov. from Hawai'i Island, and a discussion of *L. elaiophylla*
Jeffery K. Stallman, Don E. Hemmes, Nicole A. Hynson, Michael H. Shintaku 471

Notes on rust fungi in China 8. *Pucciniastrum tiliae* life cycle and new host plants inferred from phylogenetic evidence
Jing-Xin Ji, Zhuang Li, Yu Li, Makoto Kakishima 490

Guayaquilia gen. nov., typified by *Idriella cubensis*
Freddy Magdama, Daynet Sosa, Fernando Espinoza, Lizette Serrano, Simón Pérez-Martinez, Elaine Malosso, Margarita Hernández-Restrepo, Rafael F. Castañeda-Ruiz 501

Distoseptispora longispora sp. nov. from freshwater habitats in China
Hai-Yan Song, Aly Farag El Sheikha, Zhi-Jun Zhai, Jian-Ping Zhou, Ming-Hui Chen, Guang-Hua Huo, Xi-Gen Huang, Dian-Ming Hu 513

Notes on rust fungi in China 9. *Puccinia miscanthi* life cycle and morphology confirmed by inoculation
Jing-Xin Ji, Zhuang Li, Yu Li, Makoto Kakishima 525

Biodiversity of heat-resistant ascomycetes from semi-arid soils in Argentina
Stella Maris Romero, Andrea Irene Romero, Alberto Miguel Stchigel, Ernesto Rodríguez Andrade, Viviana Andrea Barrera, José Francisco Cano, Ricardo Comerio 535

Cortinarius rapaceoides, a new record for Turkey
Meryem Senay Şengul Demirak & Hakan Işık 559

New records of *Toninia* from China
Congcong Miao, Meijie Sun, Xiao Zhang, Zhaojie Ren, Ling Hu 569

Serendipita sacchari sp. nov. from a sugarcane rhizosphere in southern China — Ling Xie, Yan-Yan Long, Yan Zhang, Yan-Lu Chen, Wen-Long Zhang 579

Five *Nolanea* spp. nov. from Brazil — Fernanda Karstedt, Sarah E. Bergemann, Marina Capelari 589

First record of *Trappea darkeri* from Turkey — Yasin Uzun, Osman Berber, Abdullah Kaya 613

Mesocorynespora sinensis gen. & sp. nov. from southern China — Zhao-Huan Xu, Kai Zhang, You-Qiang Luo, Xiu-Guo Zhang, Rafael F. Castañeda-Ruíz, Jian Ma 617

Corynesporopsis hainanensis sp. nov., a bambusicolous fungus from southern China — Zhao-Huan Xu, Xu-Gen Shi, Wei-Gang Kuang, Xiu-Guo Zhang, Rafael F. Castañeda-Ruíz, Jian Ma 623

Hymenochaete longisterigmata sp. nov. from India — Navpreet Kaur, Avneet Pal Singh, Gurpaul Singh Dhingra 631

Entoloma conferendum, Hygrocybe coccineocrenata, and *Hypholoma ericaeum* new to Montenegro — Ilinka Ćetković, Zdenko Tkalčec, Snežana Dragićević, Antun Alegro, Vedran Šegota, Margita Jadan, Neven Matočec, Ivana Kušan, Željko Zgrablić, Armin Mešić 637

First Pakistani report of *Erysiphe betae* on the invasive weed *Chenopodium ambrosioides* — Ayesha Anwar, Najam ul Sehar Afshan, Aamna Ishaq, Maria Riaz, Abdul Nasir Khalid, Siraj Uddin 649

Crustose lichens new to India — Rupjyoti Gogoi, Siljo Joseph, Manas Pratim Choudhury, Sanjeeva Nayaka, Farishta Yasmin 657

Chromelosporium re-evaluated, with *Chromelosporiopsis* gen. nov. and *Geohypha* stat. nov. — Grégoire L. Hennebert 665

publication date for volume one hundred thirty-five (2)

MYCOTAXON *for* April–June 2020 (i–xiv + 235–471)
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Nomenclatural novelties and typifications proposed in Mycotaxon 135(3)

Botrytis isabellina Preuss (lectotypified)
[MBT 392406], p. 671

Chromelosporium herbicola (Ellis & Dearn.) Hennebert
[MB 833990], p.673

Chromelosporiopsis Hennebert
[MB 835622], p. 688

Chromelosporiopsis carnea (Schumach.) Hennebert
[MB 835623], p. 689

Chromelosporiopsis coerulescens (Bonord.) Hennebert
[MB 835625], p. 695

Corynesporopsis hainanensis Z.H. Xu, Jian Ma, X.G. Zhang & R.F. Castañeda
[MB 836568], p. 624

Distoseptispora longispora H.Y. Song & D.M. Hu
[MB 826916], p. 516

Geohypha (Fr.) Hennebert
[MB 835628], p. 708

Geohypha terrestris (Fr.) Hennebert
[MB 835629], p. 709

Guayaquilia R.F. Castañeda, Magdama, D. Sosa & Hern.-Restr.
[MB 831849], p. 506

Guayaquilia cubensis (R.F. Castañeda & G.R.W. Arnold) R.F. Castañeda, Magdama, D. Sosa & Hern.Restr.
[MB 831851], p. 507

Hymenochaete longisterigmata Nav. Kaur, Avn.P. Singh & Dhingra
[MB 832678], p. 632

Lepiota punaensis Stallman
[MB 832104], p. 477

Mesocorynespora Jian Ma, X.G. Zhang & R.F. Castañeda
[MB 836511], p. 618

Mesocorynespora sinensis Jian Ma, X.G. Zhang & R.F. Castañeda
[MB 836512], p. 618

Nolanea albertinae Karstedt & Capelari
[MB 826784], p. 596

Nolanea atropapillata Karstedt & Capelari
[MB 805603], p. 600

Nolanea pallidosalmonea Karstedt & Capelari
[MB 805604], p. 602

Nolanea parvispora Karstedt & Capelari
[MB 805605], p. 603
Nolanea tricholomatoidea Karstedt & Capelari
[MB 805607], p. 606
Polyactis carnea Ehrenb. (lectotypified)
[MBT 392408], p. 689
Puccinia miscanthi Miura (neotypified)
[MBT 388544], p. 531
Serendipita sacchari L. Xie, Y.Y. Long & Y.L. Chen
[MB 836761], p. 582
Trichoderma laeve Pers. (lectotypified)
[MBT 392413], p. 709

Reviewers — volume one hundred thirty-five (3)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this issue.

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Corrigenda for Mycotaxon 135–(3)

Cited below are mistakes present in files submitted for PDF conversion in the current issue but not detected by the authors until after the PDF had been processed.

p. 495, line 10	for: A–D	read: 5A–D
p. 496, line 13	for: Helongjiang	read: Heilongjiang
p. 531, line 8	for: Helongjiang	read: Heilongjiang
p. 533, line 13	for: **Wuhan**	read: **Wuchang**

From the Editor-in-Chief

Who 'authors' science?—Fungal research is now so specialized that the single-authored paper has become a rare beast indeed. Modern mycological systematics now rely on contributions from masters from several different disciplines: alpha taxonomists and collectors well versed in morphological identification, ecologists able to link environmental requirements and taxa, physiologists and "beta" taxonomists who craft diagnostic matrices from colonies in Petri dishes, "gene jockeys" who reliably deliver pristine sequences from targeted DNA regions, and evolutionary biologists wielding Bayesian analysis and Markov chain Monte Carlo methods with morphological analytics to generate elegant and reliable phylogenies.

To these may be added those who summarize, analyze, and wrap everything up in a research paper—the authors. In multi-authored papers, there is usually one individual who "drives" the paper, although sometimes two authors may be said to "contribute equally." The first-named (also called "senior") authors should have participated in the research since its inception so that they understand all aspects well enough to create a clear and comprehensible presentation of their research. Unfortunately lists begin with one name, and often the author with the best English writing abilities ascends to first position by default, irrespective of the number of scientific contributions made during research. Alternately, the most fluent English speakers who are designated corresponding authors may appear anywhere in the lists. There is also the trend toward 'anchoring' authorship by placing the Lab supervisor in last place; here we devoutly hope that the lab head has read the paper in its entirety and corrected mistakes well before journal submission.

Your editors recognize the problems encountered by contributing scientists in deciding which contributors to include as authors and which to recognize in the Acknowledgments. We definitely expect all authors to gather frequently to discuss the research while in progress, when being written, and when being prepared for submission. These discussions are true teaching moments, and authors who have eliminated mistakes during these stringent vetting processes will have much better success in publishing their science.

Mycotaxon art—Your editors are unusually fortunate to be able to present on our cover such beautiful art created by authors to illustrate their own research. The illustrations featured on our covers are selected from papers proposing taxa new to science. However, there are some truly spectacular plates in this issue accompanying previously named taxa that "escaped" cover selection. Part of the pleasure of opening a new Mycotaxon is leafing through the beautiful fungal portraits lurking inside.

Mycotaxon 135(3) contains 18 papers by 92 authors (representing 17 countries) as revised by 32 expert reviewers and the editors.

The 2020 July–September Mycotaxon proposes four new genera (*Chromelosporiopsis, Geohypha, Guayaquilia,* and *Mesocorynespora*) and ten species new

to science representing *Corynesporopsis, Distoseptispora, Mesocorynespora,* and *Serendipita* from China; *Hymenochaete* from India; *Lepiota* from Hawai'i (U.S.A.); and *Nolanea* from Brazil. We also offer five new combinations in *Chromelosporium, Chromelosporiopsis, Geohypha,* and *Guayaquilia* and four newly registered typifications for *Botrytis, Polyactis, Puccinia,* and *Trichoderma.*

New species range extensions are reported for [ascomycetes] five heat resistant species in an elegant culture study from Argentina; [basidiomycetes] *Cortinarius* & *Trappea* in Turkey and *Entoloma, Hygrocybe,* and *Hypholoma* in Montenegro; and [lichens] *Bacidia, Malmidea, Porina,* and *Pyrenula* in India and *Toninia* in China. New hosts are cited for *Erysiphe* in Pakistan and *Pucciniastrum* in China. We also offer keys to species of *Lepiota* in Hawai'i and *Toninia* s.l. in China along with worldwide keys to *Corynesporopsis, Distoseptispora,* and *Mesocorynespora*.

The July–September issue now offers more molecularly supported taxonomic studies: nine new species representing *Distoseptispora, Guayaquilia, Lepiota, Nolanea,* and *Serendipita*), one *Cortinarius* range extension, an *Erysiphe* host association, and a full life cycle of the rust *Pucciniastrum tiliae* are all supported by phylogenetic analyses, while another life cycle is established for *Puccinia miscanthi* via culture studies.

Alpha taxonomy is not dead, particularly when supported by a lifetime of close microscopical scrutiny and systematic research. The issue ends with "my final paper" by our co-founding editor, Gregoire Hennebert, who presents a masterful synopsis of a difficult (and often overlooked) group of fungi and concluding with a warm memorial of Agriculture Canada's irreplaceable Stan Hughes, who died last year at the age of 102.

Warm regards,
Lorelei L. Norvell (*Editor-in-Chief*)
9 October 2020

2020 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2020 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. This page briefly summarizes our '4-step' submission process.

1—Peer review: Authors first contact peer reviewers (two for journal papers; three for mycobiota/fungae) before sending them formatted text & illustration files and the appropriate 2020 Mycotaxon journal or mycota reviewer comment form. Experts return revisions & comments to both the *Editor-in-Chief* <editor@mycotaxon.com> and authors. All co-authors must correct and *proof-read* their files before submitting them to the *Nomenclature Editor*.

2—Nomenclatural review: Authors email all **error-free** text & illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz>. Place **first author surname** + **genus** + 'Mycotaxon' on the subject line, and (required) attach a completed submission form. The Nomenclature Editor will (i) immediately assign the accession number and (ii) after a few weeks return his notes and suggested revisions to the author(s) and *Editor-in-Chief*.

3—Final submission: All coauthors thoroughly revise and proof-read files to prepare error-free text and images ready for immediate publication. Poorly formatted copy will be rejected or returned for revision. E-mail the final manuscript to the *Editor-in-Chief* <editor@mycotaxon.com>, adding the **accession number** to the message and **all** files, which include a **(i)** revised 2020 submission form, all **(ii)** text files and **(iii)** jpg images, and **(iv)** FN, IF, or MB identifier verifications for each new name or typification. The *Editor-in-Chief* acknowledges submissions within two weeks of final submission but requests authors to wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review & publication: The *Editor-in-Chief* conducts a final grammatical and scientific review and returns her editorial revisions to all expert reviewers and coauthors for final author approval. Author-approved files are placed in the publication queue.

The PDF proof and bibliographic & nomenclatural index entries are sent to all coauthors for final inspection. After PDF processing, the *Editor-in-Chief* corrects only PDF editorial/conversion and index entry errors; corrections of all other errors are listed in the Errata of a subsequent issue for no charge. Authors will pay fees for mycobiota uploads, optional open access, and correction of major author errors to the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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Lepiota punaensis sp. nov. from Hawai'i Island, and a discussion of *L. elaiophylla*

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Abstract—A new species, *Lepiota punaensis*, in *L.* subsect. *Helveolinae* is described from the Puna District of Hawai'i Island. A species closely resembling *L. elaiophylla* in morphology but genetically distant from the species paratype is also discussed. Both taxa and additional Hawaiian collections are analyzed in an nrITS phylogeny, and a key to *Lepiota* on Hawai'i Island is given.

Key words—*Agaricaceae*, *Casuarina equisetifolia*, cryptic species

Introduction

Lepiota (Pers.) Gray is a large genus of more than 400 species (Kirk & al. 2008) with widespread distribution that continues to see new species described regularly from temperate (Caballero & al. 2015), subtropical (Qasim & al. 2015), and tropical (Sysouphanthong & al. 2016) environments. Exceptions occur, but in general the genus is characterized macroscopically by white to yellow-spored agarics with free gills, evidence of partial and universal veils, but lacking a volva. Microscopically the lamellar trama are regular, and

basidiospores are thin-walled, without a germ pore, generally dextrinoid, and not metachromatic in Cresyl Blue. *Lepiota* has historically been split into sections based on morphology (e.g., Bon 1993), and more recently, into clades based on molecular data (Vellinga 2003). *Lepiota* subsect. *Helveolinae* Bon & Boiffard [in *L.* sect. *Ovisporae* (J.E. Lange) Kühner], which contains the two species closely examined in this manuscript, is characterized morphologically by ellipsoid to oblong spores with a pileus covering composed of a trichoderm of predominantly long cylindrical elements and has been consistently recovered as monophyletic in molecular analyses of *Lepiota* and its infrageneric sections (Vellinga 2003; Qasim & al. 2015, 2016; Sysouphanthong & al. 2012, 2016; Liang & al. 2018; but see Liang & al. 2011). This clade contains species such as *Lepiota subincarnata* J.E. Lange and *L. brunneoincarnata* Chodat & C. Martín, which are known to contain amatoxins (Benjamin 1995).

Our project documenting the lepiotaceous fungi from Hawai'i Island reports a new taxon from *L.* subsect. *Helveolinae*, *Lepiota punaensis*, and a new record for Hawai'i, *Lepiota* cf. *elaiophylla*. In addition, *Lepiota aspera* (Pers.) Quél., *Lepiota besseyi* H.V. Sm. & N.S. Weber, *Lepiota pseudorubella* Gubitz, *Lepiota rubrobrunnea* Gubitz, and a poorly known taxon with an epithelial pileus covering (MK412575) are confirmed as occurring on Hawai'i Island. All species are found in wet, lowland environments associated with alien vegetation and are therefore presumed to be introduced.

Materials & methods

Collection and morphologic description

Basidiome collecting occurred from September 2016 to January 2019 on Hawai'i Island. Photographs were taken in the field with a Canon Rebel T4i camera and have not been altered (Plates 2a; 3a). Morphological details were recorded at the time of collection. Descriptions of Hawaiian habitats and vegetation zones are from Gagné & Cuddihy (1999). Macroscopic descriptions are based on terminology from Vellinga (2001) and color descriptions are from Kornerup & Wanscher (1978). After collection, specimens were desiccated before microscopic and molecular analysis, and are deposited at the Joseph F. Rock Herbarium, University of Hawaii at Mānoa, Honolulu, HI, USA (HAW).

Measurement of microscopic structures was performed on material rehydrated in 10% ammonia (pileus and stipe covering, spores), or 10% ammonia with 1% Congo Red (all other structures). Melzer's reagent, distilled water, and 1% Cresyl Blue in distilled water were also used to observe natural colors and color-changing reactions. Mature spores were measured in side view (excluding the hilar appendage) using the program Piximètre version 5.9 (http://ach.log.free.fr/Piximetre/). Measurements represent values falling within a 95% confidence

interval; the annotation [30, 4, 3] indicates that thirty spores from four specimens in three collections were measured. Micrographs for import to Piximètre were captured using an Olympus XM10 microscope camera attachment on an Olympus BX53 microscope with the software cell-sens Dimension version 1.5.

Molecular analysis

The primers ITS1F and ITS4 were used to amplify the nuclear ribosomal internal transcribed spacer region (nrITS; Schoch & al. 2012) via the polymerase chain reaction from dried fungal tissue of eleven specimens from Hawai'i Island. Amplicons were Sanger sequenced in both forward and reverse directions using the primers ITS1F and ITS4, and sequences were trimmed, edited, and forward and reverse reads aligned into consensus sequences in Geneious v. 9.1.8 (Kearse & al. 2012). Sequences generated were further investigated using the BLAST tool in GenBank (NCBI 2015), and the top sequences by similarity to Hawaiian taxa, as well

Table 1. Sequences newly generated and downloaded from GenBank for phylogenetic analysis.

taxon (collection #)	GenBank #	taxon (collection #)	GenBank #
C. hetieri	AY176459	*L. luteophylla*	AY176475
C. pulverulenta	AF391035	*L. magnispora*	AF391005
C. seminuda	JF907983	*L. pilodes*	AY176476
C. sp. (Hawai'i) (JKS 143)	**MK412600**	***L. punaensis* (JKS 64)**	**MK412572**
C. sp. (Hawai'i) (JKS 140)	**MK412604**	***L. punaensis* (JKS 86)**	**MK412577**
***Ch. molybdites* (JKS 96)**	**MK412591**	***L. punaensis* (JKS 87)**	**MK412578**
L. andegavensis	KP004931	***L. rubrobrunnea* (JKS 118)**	**MK412583**
L. apatelia	AY176462	*L. rhodophylla*	AY176480
L. aspera	KP843884	**L. sp. (O'ahu) (SS 1)**	**MK412617**
L. aspera	**MK412598**	*L.* sp. (Australia)	KP012693
L. brunneoincarnata	FJ998395	*L.* sp. (Australia)	KP012854
L. castanea	AY176463	**L. sp. (Hawai'i) (JKS 74)**	**MK412575**
L. castaneidisca	AF391061	**L. sp. (Kaua'i)**	**AY176402**
L. cristata	U85327	*L.* sp. (Thailand)	HQ647293
L. cf. *elaiophylla*	**MK412581**	*L.* sp. (Thailand)	JN224825
L. cf. *elaiophylla* (JKS 71)	**MK412589**	*L.* sp. (Thailand)	JN224826
L. cf. *elaiophylla* (JKS 77)	**MK412588**	*L.* sp. (Thailand)	JN224828
L. elaiophylla	MH979467	*L. subincarnata*	AY176491
L. elaiophylla	AF391024	*L. thiersii*	AY176492
L. erminea	AY176470	*L. tomentella*	EF080868
L. farinolens	AY176368	*L. vellingana*	HE974764
L. felina	U85330	*L. xanthophylla*	AY176405
L. helveola	MH979466	*M. haematospermum*	KF953545
L. himalayensis	HE614898	Uncultured fungus (Australia)	FJ528742

Sequences with collection numbers were newly generated; Hawaiian sequences are in **bold.**
C. = *Cystolepiota*, *Ch.* = *Chlorophyllum*, *L.* = *Lepiota*, and *M.* = *Melanophyllum*.

as representatives from each of the clades of *Lepiota* identified in Vellinga (2003) were included in our phylogenetic analyses (Table 1). Pairwise genetic distances (Table 2) were calculated in Geneious by comparing aligned, overlapping regions, excluding gaps and ambiguous positions. *Lepiota besseyi* was excluded from our phylogenetic analysis and key to *Lepiota* because it falls outside of *Lepiota* in the *Leucocoprinus* Pat. and *Leucoagaricus* Locq. ex Singer clade in *Agaricaceae* Chevall., although a formal transfer has not been made (Vellinga & al. 2011).

Table 2. Pairwise genetic distance comparison between *Lepiota* cf. *elaiophylla* and closest *Lepiota* relatives in GenBank.

	elaiophylla (Canada) MH979467	*elaiophylla* (Netherlands) AF391024	cf. *elaiophylla* (Hawai'i) MK412581	cf. *elaiophylla* (Hawai'i) MK412581	cf. *elaiophylla* (Hawai'i) MK412588
elaiophylla (Netherlands) AF391024	92.9%				
cf. *elaiophylla* (Hawai'i) MK412581	99.9%	92.9%			
cf. *elaiophylla* (Hawai'i) MK412589	99.9%	92.9%	99.9%		
cf. *elaiophylla* (Hawai'i) MK412581	99.9%	92.9%	99.9%	99.9%	
L. sp. (Australia) KP012854	98.3%	93.2%	98.3%	98.3%	98.3%

Sequences were aligned in Geneious with the default settings of the MAFFT (Katoh & Standley 2013) plugin, then imported to Molecular Evolutionary Genetics Analysis (MEGA) version X (Kumar & al. 2018). Based on the "Find Best DNA Model" tool in MEGA, Maximum Likelihood (ML) phylogenetic trees were created with all sites with gaps and/or missing data eliminated resulting in 465 total positions in the final dataset using the following settings: General Time Reversible (GTR) model (Nei & Kumar 2000) including gamma distributed (G) and invariant sites (I), moderate branch swap filter, and Subtree-Pruning-Regrafting (Extensive, level 5) for the ML heuristic method. The initial tree was built using the default neighbor-joining/bioneighbor-joining method, and the phylogeny was tested with 1000 bootstrap replicates.

The alignment was further analyzed using the Geneious plugin MrBayes (Huelsenbeck & Ronquist 2001). The tree was constructed with a GTR model including G and I sites with default settings (four chains for 1,100,000 generations, sampled every 200 generations with the first 100,000 generations discarded as burn-in). Tree images were edited in Inkscape version 3 (https://inkscape.org/).

Results & discussion

Our Bayesian and ML phylogenetic analyses generated identical tree topologies when considering our ML tree with the highest log likelihood (–4599.22) shown in Fig. 1. The topology of our *Lepiota* phylogeny is similar to prior analyses from the last 16 years and does not differ greatly from Vellinga's (2003) work using nrITS and nrLSU sequences, Qasim & al.'s (2015; 2016) works using nrITS sequences, Sysouphanthong & al.'s (2012; 2016) works using nrITS sequences, and Liang & al.'s (2018) work using nrITS, nrLSU, IGS, and mtSSU sequences (but see Liang & al. 2011 using nrITS, nrLSU, IGS, and mtSSU sequences where species with a trichoderm of long elements in clade 2 were dispersed).

Bootstrap support values for clades 2 and 4 (as defined by Vellinga 2003) are too low to draw general conclusions about the deeper relationships of our Hawaiian taxa within the genus, but our species clusters do reveal interesting information about close relatives of Hawaiian *Lepiota* s.l. species.

Lepiota punaensis is always on a long branch by itself with the closest nrITS match in GenBank an 87% similar Australian sequence (KP012854). *Lepiota punaensis* is sister to the cluster of species from Hawai'i, Canada, Australia, and Europe, all of which (except the Australian collection) are morphologically identified as *L. elaiophylla.* The Hawaiian *L.* cf. *elaiophylla* is genetically identical (99.9% similar, Table 2) to a collection identified as *L. elaiophylla* from a greenhouse in Canada, close to (98.3% similar, Table 2) a collection from a native *Eucalyptus* and *Acacia* swamp in Australia (M. Barrett, pers. comm.); these three geographically separated collections form a clade sister to the paratype of *L. elaiophylla* from the Netherlands, where the species was originally described. The collection from the Netherlands is 92.9% similar to the Hawaiian collections based on our alignment (Table 2).

Regarding other Hawaiian collections with a trichoderm of predominantly long elements, *L. rubrobrunnea* is on a long branch in a poorly supported group and its closest relatives are unclear. A *Lepiota* sp. collected on the UH Mānoa Campus on O'ahu clusters in a well-supported clade with *L. vellingana* Nawaz & Khalid, currently known from Pakistan (Nawaz & al. 2013), and *L. farinolens* Bon & G. Riousset, from France (Bon 1992). A species collected on the Island of Kaua'i clusters in a well-supported clade with three taxa from Thailand and *L. subincarnata*, a species known from Europe, North America, and South Asia (Razaq & al. 2014). It may be conspecific with sequence JN224826, a species collected in Chiang Mai Province in 2008 that, according to notes in GenBank, is "close to *L. subincarnata*."

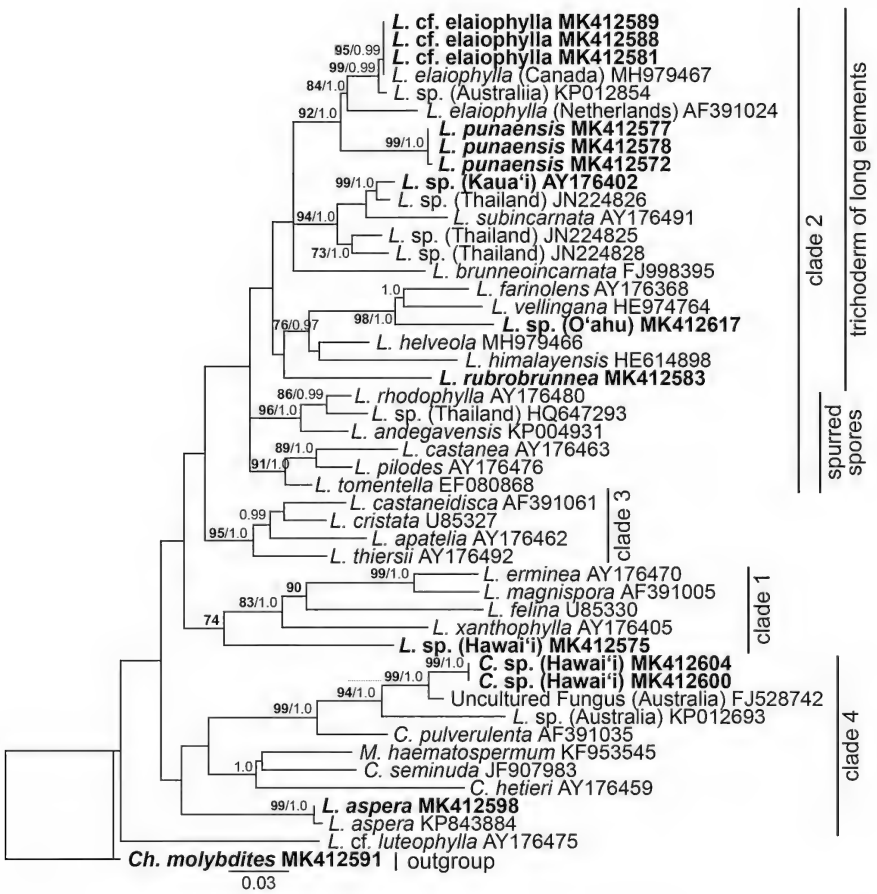

Fig. 1. Phylogenetic analysis of selected sequences and species of *Lepiota* based on nrITS sequences. The phylogeny was inferred using the Maximum Likelihood method and is drawn to scale, with branch lengths measured in the number of substitutions per site. Maximum Likelihood bootstrap support values ≥70% are shown in bold, and Bayesian posterior probabilities ≥0.95 are shown in normal type. Hawaiian taxa are in bold, and islands are indicated for taxa not identified to species. *C.* = *Cystolepiota*, *Ch.* = *Chlorophyllum*, *L.* = *Lepiota*, and *M.* = *Melanophyllum*.

The Hawaiian *Cystolepiota* species clusters in a well-supported clade with two unknown taxa from Australia, and these are sister to *Cystolepiota pulverulenta* (Huijsman) Vellinga [= *C. petasiformis* (Murrill) Vellinga], a purportedly widespread species found in Europe (Vellinga 2001), western North America (Vellinga 2006), and India (Kumar & Manimohan 2009). The closest Australian sequence (FJ528742) is 96% similar to the Hawaiian

Cystolepiota species, and its lack of any closer matches in GenBank and association with native vegetation supports the inference that it is a Hawaiian endemic (Hemmes & Desjardin 2002). A poorly known *Lepiota* (MK412575) that was only collected once in this study is on its own long branch in clade 1 (with only 74% support), and its closest relatives are unclear.

There are few data at this time to support broader conclusions about the origins and full ranges of Hawaiian *Lepiota* s.l. taxa. *Lepiota pseudorubella* lacks molecular data, *L. rubrobrunnea* and *L.* sp. (MK412575) have uncertain placement in our tree due to low support values, and *L.* sp. (MK412617) is on a relatively long branch in a clade with dispersed taxa. *Lepiota aspera* and *Melanophyllum haematospermum* (Bull.) Kreisel (for which no Hawaiian sequences are available) have widespread distributions, and *L. pseudorubella*, *L. rubrobrunnea*, and *L.* cf. *elaiophylla* have all been collected in artificially warm environments in temperate areas such as Canada and Germany (Gubitz 2008). The only putatively native Hawaiian taxon, *Cystolepiota* sp., is likely of Australian origin, while a collection from Kaua'i (AY176402) clusters with several species from Thailand. *Lepiota punaensis* and *L.* cf. *elaiophylla* have a close relative found in native Australian vegetation, but this is far from conclusive in establishing a location of origin or theory of dispersal.

Taxonomy

Lepiota punaensis Stallman, **sp. nov.** PLATE 2

MB 832104

Differs from *Lepiota elaiophylla* by its white lamellae and from *L. subincarnata* by its pileus covering containing short and narrowly clavate cells.

TYPE: USA, Hawai'i, Hawai'i Island, Puna District, MacKenzie State Recreation Area, 19°26′11″N 154°51′57″W, in duff under *Casuarina equisetifolia* L. and *Cocos nucifera* L., 9 Dec 2016, coll. JKS 86 (**Holotype**, HAW-F-00256, GenBank: MK412577).

ETYMOLOGY: from the Puna District of Hawai'i Island, the only known locality of this species.

PILEUS 8–24 mm broad, at first hemispherical, becoming obtusely conical to obtusely broadly conical, then convex to plano-convex in age; pileus covered in a dense mat of warm brown (6E6 to 7E4–5; occasionally 7F6) fibrils when young, quickly breaking into minute to small scales, composed of generally appressed aggregated fibrils, over a white to off-white (6B2) background as pileus expands, fibrils denser towards the cracked, or remaining intact disc surface, lessening towards margin, margin aspect straight, shape entire to eroded in age, often with remnants of the pileus covering adhering when young, dry, non-hygrophanous; CONTEXT thin (≤3 mm), white, unchanging;

LAMELLAE, L = 36, l = 1–3, free, close, subventricose to ventricose, ≤3 mm broad, white to off-white, edge even to eroded in age; STIPE 14–35 × 1–3 (–4 at base) mm, cylindrical, equal to tapering upwards, off-white to light brown (6B2, 8C2), covered with aggregated fibrils of similar appearance to pileus covering on lower 1/2 to 1/3 of stipe that are easily removed and become less conspicuous in age, appearing glabrous above this zone, but usually with thin covering of white hyphal strands oriented parallel to the stipe (lens), also easy to remove; stipe darkening over time to brown (6F5) where handled; CONTEXT thin, concolorous with exterior of stipe, stuffed with white hyphae when young, hollow in age, base equal to slightly enlarged; BASAL TOMENTUM absent to inconspicuous; RHIZOMORPHS absent or present, white; ANNULUS present only as annular zone; SPORE DEPOSIT white; EDIBILITY toxic, likely contains amatoxins; ODOR none to fungal; TASTE none to mildly fungal.

BASIDIOSPORES [94, 6, 4] (5.4–)6.3–6.6(–7.5) × (3.2–)3.7–3.8(–4.4) µm [x = 6.4 × 3.8 µm], Q = (1.4–)1.6–1.8(–2.0) [Qm = 1.7], ellipsoid to subcylindrical (generally oblong) in side view, usually with a straight adaxial (suprahilar depression absent) and convex abaxial side; oblong to rarely obovoid in frontal view, hyaline in H_2O and NH_4OH, dextrinoid, congophilous, weakly cyanophilous, non-metachromatic in Cresyl Blue, germ pore absent, mono to less commonly biguttulate; BASIDIA [63, 6, 4] (21.2–)25.6–33.3(–34.7) × (5.9–)6.5–8(–8.3) µm [x = 28.9 × 7.2 µm], hyaline, thin-walled, narrowly clavate to subclavate, sometimes with flexuous wall, with 4 sterigmata; LAMELLAR-EDGE sterile; CHEILOCYSTIDIA [44, 6, 4] (15.6–)22.3–24.6(–31.3) × (5.1–)8.9–10.2 (–14.0) µm [x = 23.5 × 9.5 µm], narrowly clavate to more commonly clavate or broadly clavate, hyaline, thin-walled; PILEUS COVERING an intricate trichoderm of long and shorter inflated cylindrical, sometimes flexuous, golden-colored hyphae with parietal pigment, thin to slightly thick-walled, shorter elements are present and common, but not in a distinctive layer, terminal elements narrowly clavate, cylindrical, inflated cylindrical, or less commonly irregular/flexuose narrowly clavate, or with a mucronate tip, ≤160 µm long and 12 µm diam, clamp connections common; STIPITIPELLIS a cutis of tightly packed hyaline, narrowly cylindrical hyphae ≤5 µm diam, scales below annular zone similar to those on pileus but generally composed of shorter elements, above annular zone irregularly present and mostly composed of long, narrowly cylindrical, hyaline, clamped hyphae; ANNULUS as zone only; CLAMP CONNECTIONS present in all tissues.

ADDITIONAL SPECIMENS EXAMINED—**USA, Hawai'i, Hawai'i Island, Puna District,** MacKenzie State Recreation Area, 19°26′11″N 154°51′57″W, 17 Sep 2016, coll. JKS

Plate. 2. *Lepiota punaensis* (holotype, HAW-F-00256). a. Basidiomata; b. Basidiospores; c. Basidia (with four sterigmata; only two shown in this basidial view); d. Cheilocystidia; e. Stipe covering; f. Pileus covering.

64 (HAW-F-00257; GenBank MK412572); 5 Jan 2017, coll. JKS 87 (HAW-F-00258; GenBank: MK412578); 1 February 2018, coll. JKS 186 (HAW-F-00255); 2 January 2019, coll. JKS 204 (HAW-F-00259).

Ecology & distribution — Solitary to scattered on needles and seed pods of *Casuarina equisetifolia* in wet, lowland, alien environments. January, February, September, and December. Currently known only from the Puna District of Hawai'i Island.

Comments—*Lepiota punaensis* is known only from *Casuarina equisetifolia* duff in the Puna District of Hawai'i Island. The species is recognized by its small size, lack of an annulus, and small, appressed brown fibrils over an off-white cap context. Its oblong spores and pileus covering of long elements without a layer of shorter clavate elements (although shorter elements are present) place this species in *L.* subsect. *Helveolinae* with other species that contain amatoxins.

Although *L. punaensis* is a small, relatively nondescript species, few described species provide similar morphological matches, and its nrITS sequence is unique. Recent studies from around the world such as Thailand (Sysouphanthong & al. 2012), China (e.g., Liang & al. 2018), Pakistan (Qasim & al. 2016), and the Dominican Republic (Justo & al. 2015) have described new *Lepiota* species from the (neo)tropics, and have included DNA sequence data that excludes any species from these studies, or other studies incorporating nrITS sequence data, from providing a match to *L. punaensis*. Older literature can be more difficult to interpret, but a review of species sharing similarities, and newly described species without molecular data also do not reveal a close match.

Lepiota zalkavritha T.K.A. Kumar & Manim. is a species of small size in *L.* sect. *Ovisporae* described from India with no nrITS sequence (Kumar & Manimohan 2009). *Lepiota zalkavritha* differs from *L. punaensis* in having occasional apical outgrowths on its cheilocystidia, being more robust, having darker brown coloration on pileus and stipe, and growth in soil (compared to *Casuarina equisetifolia* duff). *Lepiota brevipes* Murrill is a small species described from Florida in *L.* sect. *Ovisporae* with similar coloration to *L. punaensis*, but the stipe is often much wider, and the spores are smaller at 4.1–5 × 2.5–3.1 µm (Akers 1997). *Lepiota cinnamomea* Cleland is a small species from Australia in *L.* sect. *Ovisporae* (Aberdeen 1992), but the pinkish-brown and dark-brown color descriptions of the scales and disc, and shorter elements in the pileus covering (only ≤68 µm long) rule it out. Other small species described from Australia and stated to be in *L.* sect. *Ovisporae* (Grgurinovic 1998) have annuli (e.g., *L. rimosa* Murrill) or color-changing reactions not present in *L. punaensis*.

Pegler's work from Ceylon (1972) reveals species in *L.* sect. *Ovisporae* that are much larger, have unique red or yellow colors, or have annuli that do not match *L. punaensis*, and his works from Africa (1977) and the Caribbean (1983) do not reveal close matches. Rick's (1905, 1907, 1920, 1926, 1930, 1937, 1938, 1961) works from South America present few good matches for *L. punaensis* and Pereira's (1998, 2000, 2001) present none. Rick does describe a variety of *Lepiota* species with arachnoid veils (e.g., *L. flavipes* Rick and *L. hypholoma* Rick), but other features such as the coloration or spore sizes are different.

The closest morphological match appears to be *Lepiota subincarnata*, a well-known species described from Europe that is also found in North America and Asia (Razaq & al. 2014). *Lepiota punaensis* differs in having a typically smaller pileus and containing pileus covering elements that can have flexuose walls, be narrowly clavate, and contain shorter elements (although not in a distinctive layer), all of which *L. subincarnata* lacks.

Lepiota* cf. *elaiophylla Vellinga & Huijser PLATE 3

PILEUS (15–)25–40(–50) mm broad, at first paraboloid to narrowly hemispherical, then convex, expanding to plano-convex or applanate in age, completely covered in warm brown to dark brown (7F5, 8F4, 8F6, 8F8, 9F5) fibrils when young, quickly breaking up as the pileus expands into small to medium, generally appressed scales of aggregated fibrils over a dull yellow (3A4, 3B5, 4A3, 4A2) background; fibrils concentrated at disc (sometimes remaining unbroken) and gradually lessening towards margin to reveal more of surface; margin aspect straight, shape entire to eroded in age, sometimes with medium to large remnants of pileus covering adhering to margin, non-hygrophanous, dry; CONTEXT ≤5 mm thick, yellow (3A3, 3A4, 4B4, 4A3) fading to brown or reddish brown over time; LAMELLAE L = 32, l = 3 (usually), free, ventricose, up to 9 mm broad, dull yellow to sulphur yellow (3B6, 3B4), sometimes darkening in age (3A4, 4B4, 4C5); STIPE 20–40 × 3–7 mm, cylindrical (although sometimes flattening near the pileus) and equal (rarely tapering downwards), yellow (3A3, 3B4, 3A4, 4B3, 4A2, 3B4), with a clear annular zone beginning ⅔ to ¾ from the bottom of the stipe, above which the stipe appears glabrous, but remnants of the white, cortina-like partial veil generally remain, uncommonly aggregating into larger visible structures, below covered in medium to large, horizontally-oriented brown scales, generally appressed, but more uplifted than those on the pileus, concolorous with those on the pileus; CONTEXT fleshy-fibrous, color as in pileus or slightly lighter; when young solid to stuffed with translucent to

white hyphae, hollow in age; RHIZOMORPHS present, white; BASAL TOMENTUM generally present; SPORE DEPOSIT white; EDIBILITY toxic, likely contains amatoxins; ODOR none to mildly fungal; TASTE none to mild.

BASIDIOSPORES [150, 7, 5] (5.8–)7.0–7.2(–8.4) × (3.3–)3.7–3.8(–4.2) µm [x = 7.1–3.8 µm], Q = (1.5–)1.9(–2.3) [Qm = 1.9], narrowly ellipsoid to subcylindrical (usually oblong) in side view with a convex abaxial side and straighter adaxial side (mild suprahilar depression rarely present), oblong to subcylindrical in face view, hyaline in H_2O and NH_4OH, dextrinoid, congophilous, weakly cyanophilic to cyanophilic and non-metachromatic in Cresyl Blue, germ pore absent, mono- or (less commonly) bi-guttulate; BASIDIA [57,7,5] (20.6–)23.1–28.7(–34.6) × (5.7–)7.2–8.8(–9.2) µm [x = 26 × 8 µm], narrowly clavate to clavate, hyaline, thin-walled, 4-spored, rarely 2-spored; LAMELLAR-EDGE sterile; CHEILOCYSTIDIA [39, 5, 4] (15.5–)20.1–30.1(–39.3) × (5.2–)6.1–9.8(–10.3) µm [x = 24.5 × 7.9 µm], generally narrowly clavate to clavate, but also cylindrical and somewhat flexuous, sometimes with an apical extension, narrowly utriform, or less commonly subconical, often septate with one to two short, cylindrical basal cells, with or without clamp connections, hyaline, thin-walled; PLEUROCYSTIDIA absent; PILEUS COVERING an intricate trichoderm of mostly long erect and repent, generally cylindrical hyphae with brown intracellular and parietal pigments, lacking a layer of short clavate elements, terminal cells 25–240 × 4–15 µm, most commonly 60–100 µm in length, inflated cylindrical to narrowly clavate, often flexuous; STIPITIPELLIS a cutis of narrowly cylindrical hyaline hyphae 3–7 µm diam with stipe scales below annular zone similar to scales on pileus, above this zone a covering of thin, hyaline, cylindrical, often flexuous hyphae up to 4 µm diam and 100 µm long; ANNULUS as a cortinoid zone only; rarely with stipe covering elements aggregating to create a medial to superior raised region resembling an adhering "ring"; CLAMP CONNECTIONS present in all tissues; may be absent in cheilocystidia.

SPECIMENS EXAMINED—**USA, Hawai'i, Hawai'i Island, Hilo District**, University of Hawai'i Hilo Botanical Garden, 19°42′10″N 155°04′56″W, 28 September 2016, coll. JKS 69 (HAW-F-00244); **Hilo**, 'Imiloa Astronomy Center, 19°42′07″N 155°05′16″W, 5 October 2016, coll. JKS 71 (HAW-F-00245; GenBank MK412589); 6 November 2016, coll. JKS 77 (HAW-F-00246; GenBank MK412588); **Puna District**, MacKenzie State Recreation Area, 19°26′22″N 154°51′4″W, 15 November 2017, coll. JKS 174 (HAW-F-00247).

ECOLOGY & DISTRIBUTION—Scattered to gregarious, sometimes cespitose, in wood chips, on nutrient-rich soil in disturbed settings, or on *Casuarina equisetifolia* duff in wet, lowland, alien environments. January, May, and July

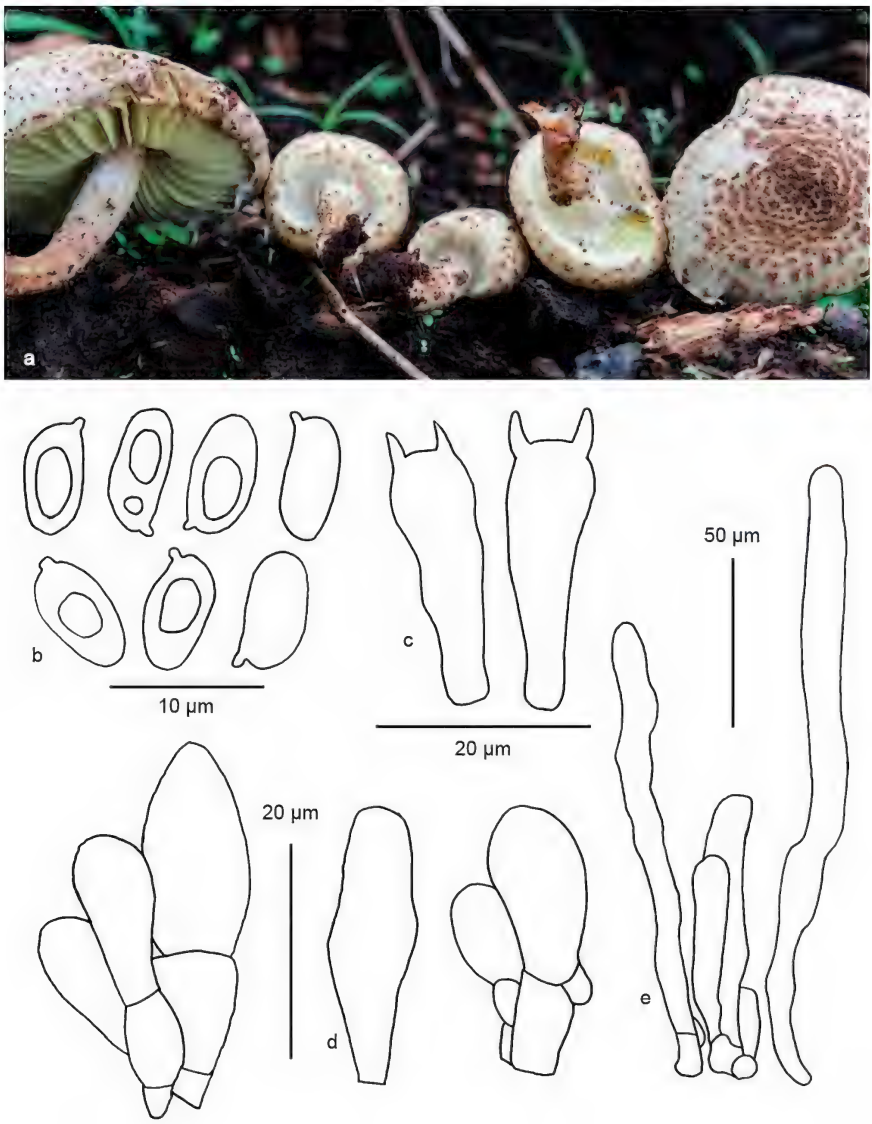

Plate. 3. *Lepiota* cf. *elaiophylla* (HAW-F-00246). a. Basidiomata; b. Basidiospores; c. Basidia (generally with four sterigmata; only two shown in these basidial views); d. Cheilocystidia; e. Pileus covering.

to December. Currently only known from the Puna and South Hilo Districts on Hawai'i Island; *L. elaiophylla* in the broad sense (see discussion) is reported

from Australia, Europe, North America, South America, India, and possibly Africa (as *L. xanthophylla* P.D. Orton).

Comments—*Lepiota* cf. *elaiophylla* occurs on wood chips, nutrient-rich soil in disturbed environments such as gardens, and under coastal *Casuarina equisetifolia* where it can fruit singly, or in large groups and cespitose clusters. Although many yellow lepiotaceous fungi occur in Hawai'i, *L.* cf. *elaiophylla* is easily distinguishable by its overall sulphur yellow coloration (including the gills) with brown scales on the pileus, robust stature (differentiating it from delicate yellow species in *Leucocoprinus*), and clear annular zone on the stipe with cortina-like partial veil remnants above a base covered in brown scales. Its oblong spores and pileus covering of primarily long elements place this species in *L.* subsect. *Helveolinae* with other species containing amatoxins.

A BLAST search reveals the top match by identity in GenBank to represent a collection identified as *Lepiota elaiophylla* by Else Vellinga, the co-describer of this species, from a greenhouse in Canada at 99.9% sequence similarity (KP012854). The next closest match (at 98.3% similarity) is MH979467 from a specimen collected near Darwin Australia growing in a native *Eucalyptus* and *Acacia* swamp habitat (M. Barrett, pers. comm.). Micrographs of the Australian collection (T. Lebel, pers. comm.) reveal spores that are more ellipsoid than in most collections of *L.* cf. *elaiophylla*, so it is unclear without additional Australian specimens whether these two taxa are morphologically identical.

Our phylogeny of *Lepiota* (Fig. 1) shows the Hawaiian *L.* cf. *elaiophylla* and the Canadian collection identified as *L. elaiophylla* as identical, with the Australian collection close on its own short branch. The Dutch paratype of *L. elaiophylla* is sister to the clade containing the Hawaiian, Canadian, and Australian collections, and a well-supported (84% bootstrap support, 1.0 Bayesian posterior probability) clade is formed when considering all taxa, including the paratype collection. See Table 2 for a comparison of pairwise genetic distances between the species discussed.

Lepiota elaiophylla was described from the Netherlands and is reported throughout Europe (e.g., Holec & Hálek 2008), Brazil (Wartchow & al. 2008, Ferreira & Cortez 2012), India (Kumar & Manimohan 2009), and possibly Africa (as *L. xanthophylla*; Pegler 1977). The Hawaiian *L.* cf. *elaiophylla* is clearly closely related to *L. elaiophylla,* from which it is macroscopically indistinguishable. The only differences are (morphologically) a more variable cheilocystidia shape in *L.* cf. *elaiophylla* and (molecularly) a different nrITS sequence, although some European collections outside the Netherlands and Brazilian collections show variation in cheilocystidia shape.

For a discussion of other *Lepiota* spp. with yellow lamellae (including the macroscopic lookalike *L. xanthophylla*, which is differentiated by containing a layer of short, clavate elements in the pileus covering and which has not been recorded from Hawai'i), see Vellinga & Huijser (1997). Collections of the European *L. xanthophylla* and *L. elaiophylla* are described with differently colored dried lamellae (yellow in *xanthophylla* and dirty olive-brown for *elaiophylla*). Collections of *L.* cf. *elaiophylla* generally have dark yellow lamellae when dried, although older, poorly dried, or damaged specimens may exhibit brown colors; so this does not appear to be a useful taxonomic feature at this time.

More information is needed to distinguish species concepts among *L.* cf. *elaiophylla*, the Australian collection, and *L. elaiophylla* from the Netherlands and throughout its reported range. One option is to argue that *L.* cf. *elaiophylla* falls within normal morphological variation (minor cystidia differences) of *L. elaiophylla*, a widespread species with high molecular variation and some morphological variation as evidenced by its presence in India (Kumar & Manimohan 2009), Brazil (Wartchow & al. 2008, Ferreira & Cortez 2012), and Australia. This option cannot be proven but falls on the conservative side of not describing a new taxon without first examining molecular data from other regions that might reveal whether there is a range of variation in the nrITS or two distinct taxa. The other option is to argue that *L.* cf. *elaiophylla* is clearly molecularly different from the Dutch *L. elaiophylla* (over 7% pairwise distance) and supported by a morphological difference (cheilocystidia shape). This argument is supported by pointing out that the related species *L. brunneoincarnata* and *L. subincarnata* initially showed little to no molecular variation across their respective geographic ranges (Europe and Central Asia; Europe, South Asia, and North America) when examined by Razaq & al. (2014).

At this time, more molecular data from Europe, South America, India (and possibly Africa) and morphological data from India and Africa are needed to clarify the species concept. The Indian collection was not given a full species description, but collections from South America reportedly have smaller spores as well as lageniform (Ferreira & Cortez 2012) or subfusoid (Wartchow & al. 2008) cheilocystidia, while collections from greenhouses in Europe primarily have narrowly clavate to clavate cheilocystidia (with the exception of the subglobose, and oval to pyriform cheilocystidia noted by Breitenbach & Kränzlin 1995). At this time, habitat does not seem to be a useful taxonomic feature as both Hawaiian and Dutch collections have been found in greenhouse

environments, although only the Hawaiian *L.* cf. *elaiophylla* is currently confirmed from non-artificial habitats in (sub)tropical environments.

Key to the *Lepiota* of Hawai'i Island

1. Pileus brownish with covering of acute pyramidal scales composed of globose or inflated elements 2
1. Pileus color variable (including brownish) with covering lacking acute pyramidal scales, at least some long (≥100 µm) cylindrical elements present 3
2. Basidiome with large membranous annulus, spores oblong to cylindrical, averaging 6.4 × 2.7 µm ..... *L. aspera*
2. Basidiome annulus and other features poorly known, spores ellipsoid to oblong, averaging 4.4 × 2.7 µm ..... *L.* sp. (MK412575; poorly known; see text)
3. Basidiome with ellipsoid spores averaging 5 × 3 µm 4
3. Basidiome with ellipsoid to oblong spores averaging 7 × 3–4 µm 5
4. Basidiome with prominent basal stipe ornamentation concolorous with pileus scales; farinaceous odor when crushed; pileus and stipe covering of long flexuose hyphae lacking abundant shorter clavate elements ..... *L. rubrobrunnea*
4. Basidiome generally lacking prominent basal stipe ornamentation, stipe scales present usually lighter than pileus scales; lacking a farinaceous odor when crushed; pileus and particularly stipe covering with abundant short clavate elements ..... *L. pseudorubella*
5. Basidiome with brown scales over a dull yellow pileus background, lamellae and stipe also yellow; arachnoid partial veil usually leaving only zone on stipe; pileus covering a trichoderm of long (≤240 µm) elements lacking shorter cells ..... *Lepiota* cf. *elaiophylla*
5. Basidiome with warm brown scales over an off-white pileus background lacking yellow coloration in all tissues; annulus a zone only; pileus covering a trichoderm of long (≤160 µm) elements with shorter, sometimes clavate hyphae interspersed but not in a distinct layer; shorter hyphae more common in stipe covering, currently known only from *Casuarina equisetifolia* duff in the Puna District of Hawai'i Island ..... *L. punaensis*

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to *Lepiota* taxonomy in general and *L. elaiophylla* in particular. We also thank Dr. Brian A. Perry (Department of Biological Sciences, California State University East Bay, USA) and Dr. Alfredo Vizzini (Department of Life Sciences & Systems Biology, University of Torino, Italy) for presubmission review.

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Notes on rust fungi in China 8. *Pucciniastrum tiliae* life cycle and new host plants inferred from phylogenetic evidence

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Abstract—The life cycle connection between spermogonial and aecial stages of a rust fungus found on *Abies holophylla* and uredinial and telial stages on *Tilia mongolica* and *T. mandshurica* collected in northeast China were confirmed by phylogenetic analyses. The rust, identified as *Pucciniastrum tiliae*, was confirmed by morphological observations. The life cycle of this rust fungus is reported for the first time in China, and *A. holophylla* and *T. mongolica* represent new host plants for the species.

Key words—phylogeny, *Pinaceae*, *Pucciniastreae*, *Tiliaceae*, tree disease

Introduction

Pucciniastrum tiliae was first described with uredinial and telial stages on *Tilia cordata* var. *japonica* [≡ *T. japonica; Tiliaceae*] collected in Hokkaido, Japan (Hiratsuka 1897). This species has been since reported on *T. amurensis* in Korea, China, and Russian Far East; *T. americana* and *T. kiusiana* in Japan; *T. mandshurica* in China and Russian Far East; *T. maximowicziana* [= *T. miyabei*] in China and Japan; *T. platyphyllos* in Japan; and *T. tuan* in China (Hiratsuka 1936, 1958; Ito 1938; Cummins 1950; Cummins & Ling 1950; Wang 1951; Kim 1963; Tai 1979; Hiratsuka & al. 1992; Azbukina 2005,

2015). The life cycle of this fungus was revealed by Kamei in Japan in 1934 through inoculations of basidiospores from teliospores on *T. japonica* and *T. maximowicziana* into needles of *Abies sachalinensis* var. *mayriana* (*Pinaceae*) (Hiratsuka & al. 1992). Although *P. tiliae* is distributed in northeast China (Tai 1979), there are no reports of a spermogonial and aecial host in the country.

During field investigation of rust fungi in northeast China, we collected uredinial and telial stages of *Pucciniastrum* on *T. mandshurica* and *T. mongolica* in the mountains of Jilin and Heilongjiang Provinces. We also observed occurrences of aecia producing long peridia on *Abies holophylla* near infected trees of *Tilia*. Therefore, we suspected life cycle connections between the rust on *Abies* and *Tilia*. Inoculation of plants by spores are an appropriate method to clarify rust life cycles (Ji & al. 2017a, b). However, collection and propagation of plants from the mountains of Maple Valley (Jilin) and Fenix National Park (Heilongjiang) where we collected specimens are strictly prohibited as they are nature conservation areas. Therefore, we applied molecular analyses to resolve these problems and to clarify the life cycle (Liu & Hambleton 2013, Ji & al. 2016, Padamsee & McKenzie 2017, Scholler & al. 2019). We report here the results of phylogenetic analyses and morphological observations of the specimens.

Materials & methods

Molecular analyses

Uredinial and telial stages on *Tilia mandshurica* and *T. mongolica* collected in mountains in Jilin and Heilongjiang Provinces, China, were used for molecular analyses. The spermogonial and aecial stages on *Abies holophylla* collected in Jiaohe, Jilin Province, China, were also used for analyses.

The total genomic DNA was directly extracted from c. 200 spores obtained from single sori on the leaf of each specimen using similar methods reported by Ji & al. (2016, 2019). Specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU); all data sequenced in this experiment were deposited in GenBank as shown in specimens examined.

The sequences obtained from specimens were aligned following Ji & al. (2019). ITS and 28S sequences related to *P. tiliae* were retrieved from GenBank and added to phylogenetic analyses (Ji & al. 2019). The accession numbers of these data are shown in the phylogenetic trees (Figs 1, 2). Phylogenetic trees were constructed using *Melampsora laricis-populina* Kleb. as outgroup according to Ji & al. (2019). The alignment and trees were deposited in TreeBASE under http://purl.org/phylo/treebase/phylows/study/TB2:S24909 (Figs 1, 2).

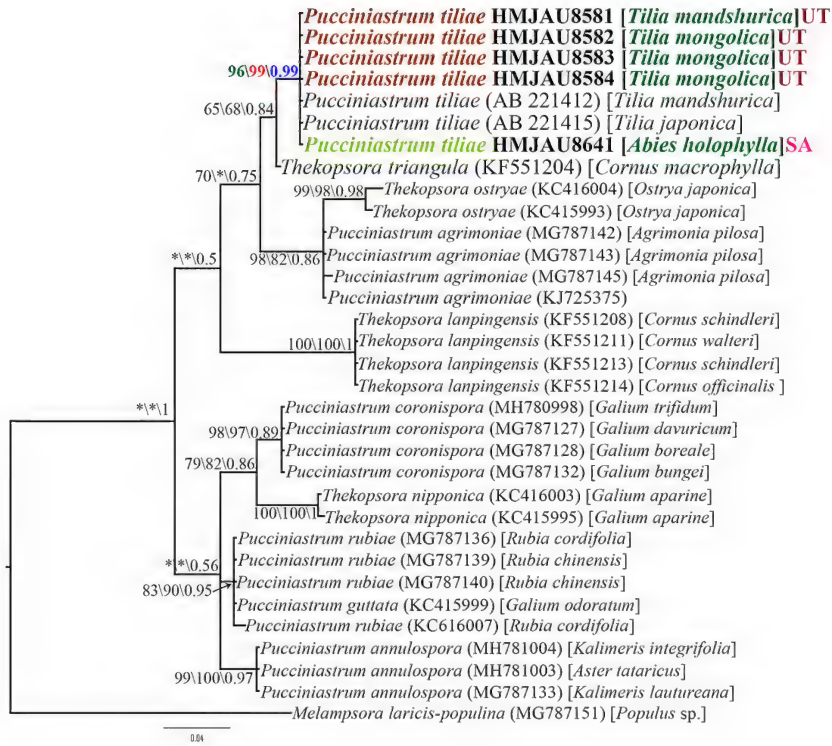

FIG. 1. Phylogenetic tree constructed by Bayesian method based 28S rDNA sequences. MP/ML bootstrap values precede Bayesian posterior probabilities (Bpp) on the nodes. Asterisks (*) indicate bootstrap values <50% or Bpp <0.5. Sample data include species name, (voucher specimen or GenBank accession number), and [host plant]. Sequences generated in this study are shown in bold face. **SA**= Spermogonial and aecial stages; **UT** = Uredinial and telial stages.

Morphological observations

Light (LM) and scanning electron (SEM) microscopy were used to examine morphological characters of rust specimens, including the size and shape of sori and spores following Ji & al. (2019).

Phylogenetic results

The 28S dataset comprised 33 sequences with 496 total characters, of which 394 were constant, 30 parsimony-uninformative variable, and 72 parsimony-informative. Parsimony analysis yielded one parsimonious tree with TL = 156, CI = 0.7692, RI = 0.9231, and RC = 0.7101. Bayesian analysis resulted in average standard deviation of split frequencies of 0.008983. The ITS dataset comprised 39 sequences of 745 total characters, of which 228 were parsimony-informative. Parsimony

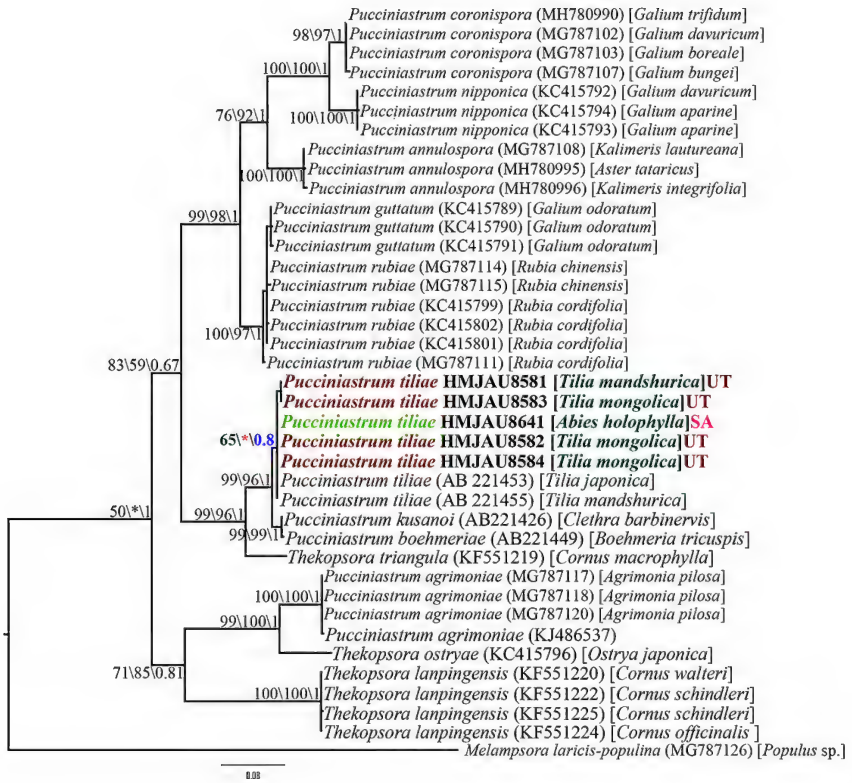
Pucciniastrum coronispora (MH780990) [Galium trifidum]
98\97\1 Pucciniastrum coronispora (MG787102) [Galium davuricum]
Pucciniastrum coronispora (MG787103) [Galium boreale]
100\100\1 Pucciniastrum coronispora (MG787107) [Galium bungei]
Pucciniastrum nipponica (KC415792) [Galium davuricum]
76\92\1 100\100\1 Pucciniastrum nipponica (KC415794) [Galium aparine]
Pucciniastrum nipponica (KC415793) [Galium aparine]
Pucciniastrum annulospora (MG787108) [Kalimeris lautureana]
100\100\1 Pucciniastrum annulospora (MH780995) [Aster tataricus]
Pucciniastrum annulospora (MH780996) [Kalimeris integrifolia]
99\98\1 Pucciniastrum guttatum (KC415789) [Galium odoratum]
Pucciniastrum guttatum (KC415790) [Galium odoratum]
Pucciniastrum guttatum (KC415791) [Galium odoratum]
Pucciniastrum rubiae (MG787114) [Rubia chinensis]
Pucciniastrum rubiae (MG787115) [Rubia chinensis]
Pucciniastrum rubiae (KC415799) [Rubia cordifolia]
100\97\1 Pucciniastrum rubiae (KC415802) [Rubia cordifolia]
Pucciniastrum rubiae (KC415801) [Rubia cordifolia]
83\59\0.67 Pucciniastrum rubiae (MG787111) [Rubia cordifolia]
Pucciniastrum tiliae HMJAU8581 [Tilia mandshurica]UT
Pucciniastrum tiliae HMJAU8583 [Tilia mongolica]UT
Pucciniastrum tiliae HMJAU8641 [Abies holophylla]SA
65*\0.8 Pucciniastrum tiliae HMJAU8582 [Tilia mongolica]UT
Pucciniastrum tiliae HMJAU8584 [Tilia mongolica]UT
99\96\1 Pucciniastrum tiliae (AB 221453) [Tilia japonica]
Pucciniastrum tiliae (AB 221455) [Tilia mandshurica]
50*\1 99\96\1 Pucciniastrum kusanoi (AB221426) [Clethra barbinervis]
99\99\1 Pucciniastrum boehmeriae (AB221449) [Boehmeria tricuspis]
Thekopsora triangula (KF551219) [Cornus macrophylla]
Pucciniastrum agrimoniae (MG787117) [Agrimonia pilosa]
100\100\1 Pucciniastrum agrimoniae (MG787118) [Agrimonia pilosa]
99\100\1 Pucciniastrum agrimoniae (MG787120) [Agrimonia pilosa]
Pucciniastrum agrimoniae (KJ486537)
Thekopsora ostryae (KC415796) [Ostrya japonica]
71\85\0.81 Thekopsora lanpingensis (KF551220) [Cornus walteri]
100\100\1 Thekopsora lanpingensis (KF551222) [Cornus schindleri]
Thekopsora lanpingensis (KF551225) [Cornus schindleri]
Thekopsora lanpingensis (KF551224) [Cornus officinalis]
Melampsora laricis-populina (MG787126) [Populus sp.]
0.05

Fig. 2. Phylogenetic tree constructed by Bayesian method based on ITS rDNA sequences. MP/ML bootstrap values precede Bayesian posterior probabilities (Bpp) on the nodes. Asterisks (*) indicate bootstrap values <50% or Bpp <0.5. Sample data include species name, (voucher specimen or GenBank accession number), and [host plant]. Sequences generated in this study are shown in bold face. **SA**= Spermogonial and aecial stages; **UT** = Uredinial and telial stages.

analysis yielded one parsimonious tree with TL = 543, CI = 0.8066, RI = 0.9417, and RC = 0.7596. Bayesian analysis resulted in average standard deviation of split frequencies of 0.003112. Tree topologies formed by MP, ML, and MCMC methods were identical among trees. The Bayesian phylogenetic trees are shown in Fig. 1 (28S) and Fig. 2 (ITS).

Both 28S and ITS phylogenetic trees included the uredinial and telial stages on *T. mandshurica* (HMJAU 8581) and *T. mongolica* (HMJAU 8582, 8583, 8584) and the spermogonial and aecial stages on *A. holophylla* (HMJAU 8641) in a monophyletic clade (Figs 1, 2). Genetically identical to one another, they can be considered the same species. The life connection between spermogonial and aecial stages on *A. holophylla* and uredinial and telial stages on *T. mandshurica* and *T. mongolica* is therefore strongly supported by the phylogenetic analyses.

Taxonomy

The phylogenetic analyses reveal that this rust fungus has a heteromacrocyclic life cycle, producing spermogonia and aecia on *Abies holophylla* (Fig. 3D) and uredinia and telia on *Tilia mandshurica* and *T. mongolica* (Figs 3A–C). LM and SEM observations showed that the morphological characteristics of spermogonial and aecial specimens on *A. holophylla* collected from the field were identical with the descriptions of *Pucciniastrum tiliae* reported by Hiratsuka (1936) and Hiratsuka & al. (1992), except for outer wall surface structure of the peridial cells (Figs 3D, 4A–C, A–D). Under SEM, these walls are minutely verrucose, in contrast to the smooth surface described by Hiratsuka & al. (1992). Previously, *P. tiliae* spermogonial and aecial stages have been reported on *Abies* species only from Japan, so this represents the first report of the *P. tiliae* life cycle from China. *Abies holophylla* is a new spermogonial and aecial host plant and occurs in northeastern China, Korea, and Russian Far East.

After clarifying the life cycle connection of *P. tiliae* with phylogenetic analyses, we described its morphology based on specimens collected in the field.

The morphological characters of uredinial and telial specimens on *T. mandshurica* and *T. mongolica* were similar to one another (Figs 3A–C, 4D–F, 5E,F) and identical to the descriptions of *P. tiliae* by Hiratsuka (1936), Hiratsuka & al. (1992) and Azbukina (2005, 2015). No researcher has previously reported *P. tiliae* on *T. mongolica* (Hiratsuka 1936, 1958; Kim 1963; Tai 1979; Hiratsuka & al. 1992; Azbukina 2005, 2015), so we add *T. mongolica* (known from Mongolia, northern China, and eastern Russia) as a new host for the rust.

Pucciniastrum tiliae Miyabe, Bot. Mag. Tokyo 11: 47, 1897. Figs 3–5

Spermogonia amphigenous, pale yellow to yellowish, subcuticular, type 3 (Cummins & Hiratsuka 2003). Aecia with long peridia, *Peridermium*-type, hypophyllous, cylindrical, orange-yellow. Peridia colorless, rupturing at the sides and apex; peridial cells ovate to ellipsoid, slightly overlapping, inner and outer walls thin, minutely verrucose. Aeciospores globose to ellipsoid, 15.0–22.0 × 13.0–16.5 µm (av. = 18.5 × 15.0 µm), walls hyaline, 1.0–2.0 µm (av. = 1.5 µm) thick, densely verrucose with annulate verrucae.

Uredinia hypophyllous, scattered to gregarious, subepidermal, small, round, covered by the epidermis, rupturing with pores, pulverulent. Peridia hemispherical, delicate, firm, rupturing at the apex, peridial cells small,

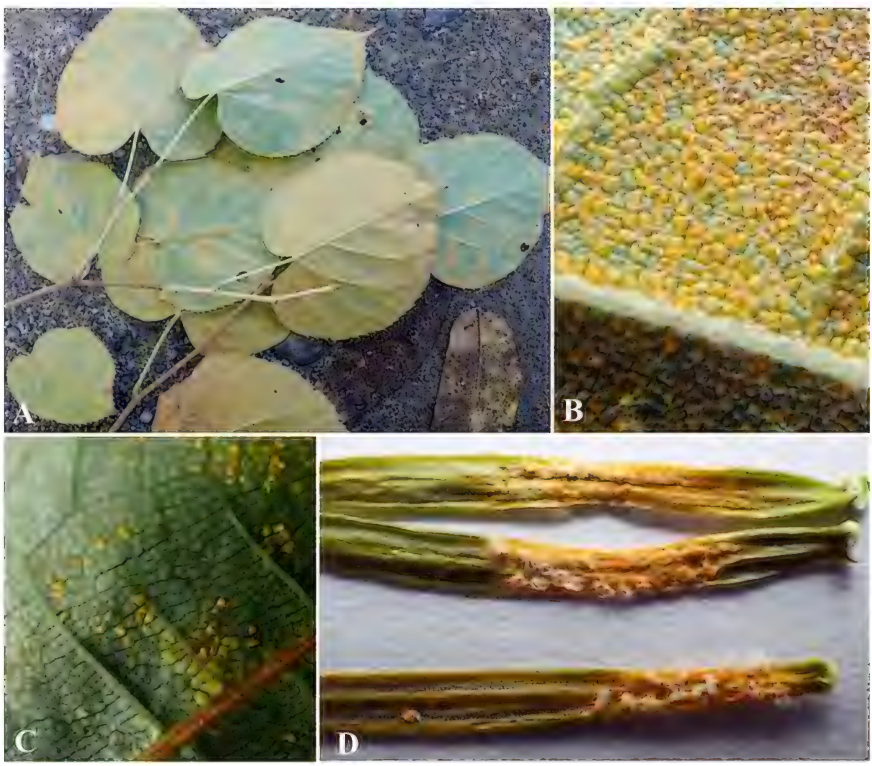

Fig. 3. *Pucciniastrum tiliae.* A. Uredinia and telia on lower leaf surface of *Tilia mongolica.* B. Telia produced on lower leaf surface of *T. mongolica.* C. Uredinia produced on lower leaf surface of *T. mongolica.* D. Aecia with long peridia produced on needles of *Abies holophylla.*

irregularly polygonal, walls smooth, thin, ostiolar cells rounded, walls smooth. Urediniospores subglobose, ellipsoid, obovate, or oblong, 16.5–25.5 × 8.0–15.5 µm (av. = 20.5 × 12.5 µm), walls echinulate, 0.5–2.0 µm (av. = 1 µm) thick. Telia hypophyllous, subepidermal, scattered or in small groups, at first orange-yellow, then brown to reddish in color. Teliospores forming under the epidermis, attached laterally, subglobose, oblong, or somewhat angular, divided into 2–6 cells by vertical or oblique septa, 21.0–37.0 × 5.5–14.0 µm (av. = 30.5 × 9.5 µm), walls thin, 0.5–1.0 µm (av. = 0.7 µm) thick, smooth.

Specimens examined—**CHINA, Jilin Province, Jiaohe**, stages 0 & I, on *Abies holophylla* Maxim., 16 June 2019 (HMJAU8641, GenBank MN294706, MG787116); stages II & III, on *Tilia mongolica* Maxim., 11 September 2017 (HMJAU8583, GenBank MG787149, MG787124; HMJAU8584, GenBank MG787150, MG787125); **Helongjiang**

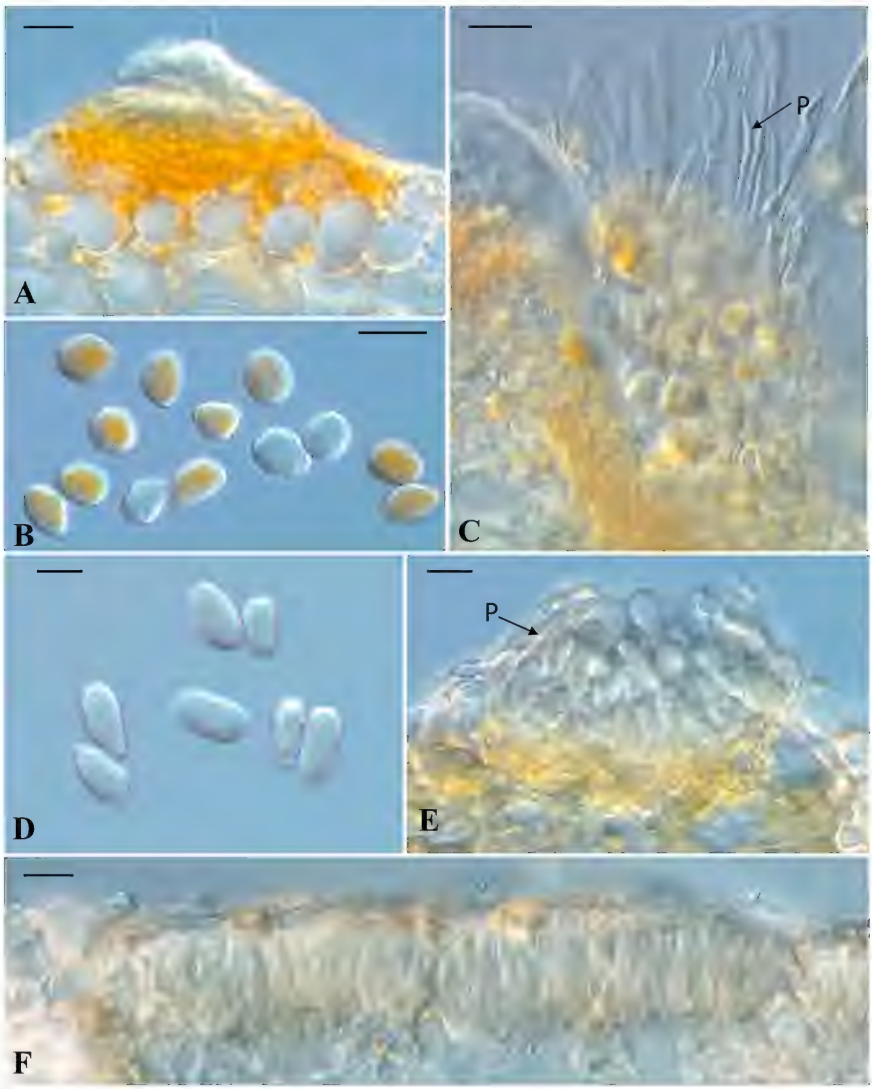

Fig. 4. *Pucciniastrum tiliae* observed with LM. A. Vertical section of spermogonium (type 3). B. Aeciospores with verrucose surface. C. Vertical section of aecium surround by peridium (P). D. Urediniospores with echinulate surface. E. Vertical section of uredinium with peridium (P), opening by central pore. F. Vertical section of telia with laterally attached teliospores, covered by host epidermis. Scale bars: A, C = 20 µm; B = 30 µm; D, E = 10 µm; F = 15 µm.

Province, Wuchang, stages II & III, on *T. mongolica*, 9 September 2017 (HMJAU8582, GenBank MG787148, MG787123); stages II & III, on *T. mandshurica* Rupr. & Maxim., 9 September 2017 (HMJAU8581, GenBank MG787147, MG787122).

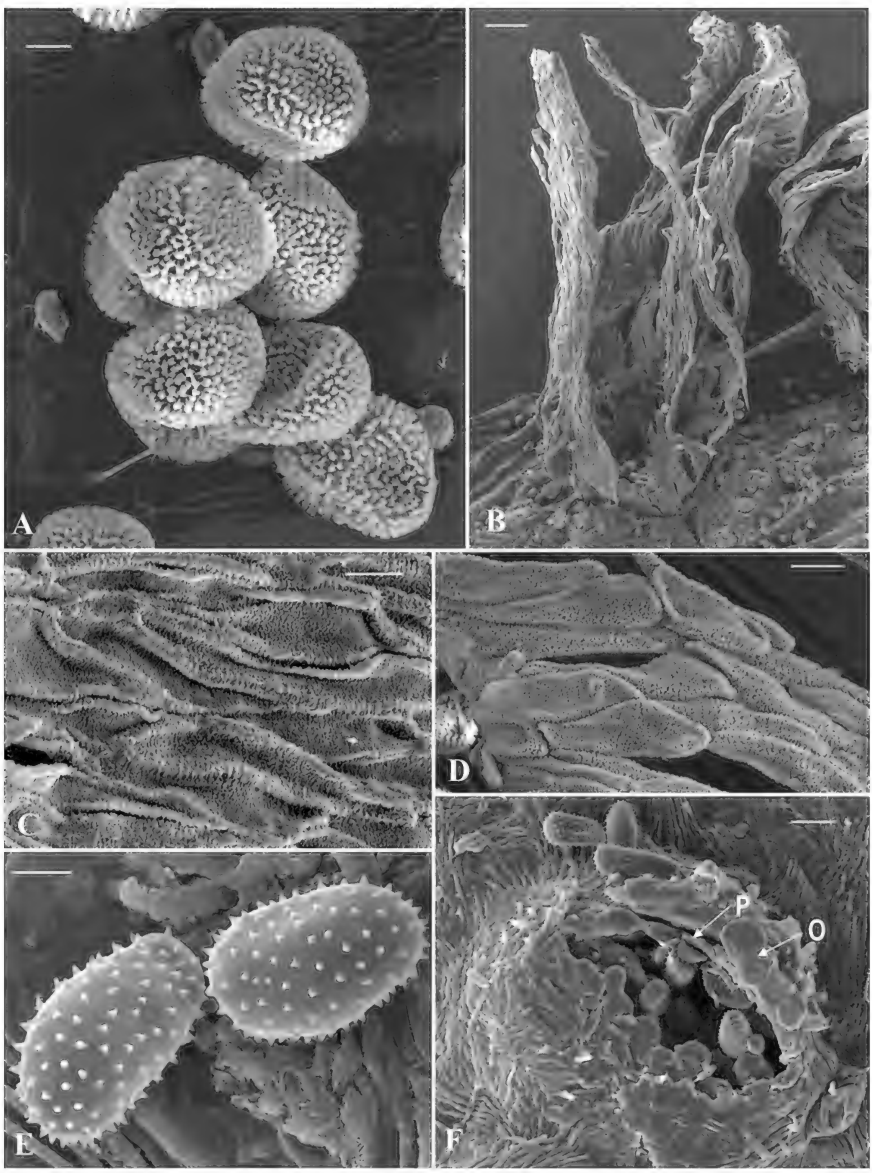

Fig. 5. *Pucciniastrum tiliae* observed with SEM. A. Aeciospores with densely verrucose surface. B. Aecium with long peridium. C. Outer walls of peridial cells. D. Inner walls of peridial cells. E. Urediniospores with echinulate surface. F. Uredinium with peridium (P) and ostiolar cells (O), opening by central pore. Scale bars: A, E = 5 μm; B = 100 μm; C, D = 20 μm; F = 10 μm.

Hosts & distribution within China—***Abies holophylla***, Jilin (new host; this paper). ***Tilia amurensis*** Rupr., Jilin (Ito 1938, Tai 1979). ***Tilia mandshurica***, Heilongjiang (Ito 1938, Hiratsuka 1958, Tai 1979). ***Tilia mongolica***, Heilongjiang, Jilin (new host; this paper). ***Tilia tuan*** Szyszył., Guizhou (Hiratsuka 1958, Tai 1979).

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Guayaquilia gen. nov., typified by *Idriella cubensis*

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Abstract—A new genus *Guayaquilia* is established to accommodate *Idriella cubensis* based on morphology and phylogenetic analysis. DNA sequence data place these specimens as incertae sedis separate from *Microdochiaceae* (*Xylariales*), forming a monophyletic lineage separated from *Neoidriella desertorum* and phylogenetically distant from *Idriella*. The novel genus is characterized by macronematous, tree-like, fasciculate, profuse dichotomously, alternately, or irregularly branched, brown conidiophores with polyblastic, denticulate, sympodial extended, intercalary and terminal conidiogenous cells that produce solitary, sublunate, subnavicular, lunate, inequilateral, (0–)1-septate, hyaline conidia.

Key words—asexual fungi, hyphomycetes, *Microdochiaceae*, taxonomy, tropics

Introduction

Idriella, introduced by Nelson & Wilhelm (1956) with *I. lunata* as type species, is distinguished by macronematous, mononematous, unbranched, and basally

inflated and apically slightly geniculate brown conidiophores, mostly reduced to polyblastic, denticulate, sympodial extended conidiogenous cells. Conidia are typically lunate or falcate, unicellular, hyaline, and acuminate towards the apex (Nelson & Wilhelm 1956). Morgan-Jones (1979) updated and provided brief nomenclatural commentaries on *Idriella australiensis*, *I. couratarii*, *I. desertorum*, *I. lunata, I. mycogonoidea, I. ramosa, I. vandalurensis,* and *I. variabilis* and proposed two new species, *I. angustispora* and *I. bambusae*. Von Arx (1981), who presented a combined key for *Idriella* and *Microdochium* species, proposed the conidial shape and the habitat as the main characters to separate the genera; he included the *Idriella* species treated by Morgan-Jones (1979), excluded *I. tropicalis* as similar to *I. variabilis*, and added five new combinations (*I. bolleyi*, *I. caespitosa, I. falcata, I. grisea*, and *I. tainanensis*). Subsequently, 16 new species have been described *I. acerosa, I. amazonica, I. angamosensis, I. asaicola, I. cagnizarii, I. campnospermae, I. cubensis, I. euterpes, I. jambosae, I. licualae, I. mycophila, I. rara, I. rhododendri, I. setiformis, I. stilboidea,* and *I. uncinospora* (Castañeda-Ruiz 1985, 1986; Castañeda-Ruiz & Arnold 1985; Castañeda-Ruiz & Kendrick 1991; Castañeda-Ruiz & al. 1997; Matsushima 1985, 1995; Rodrigues & Samuels 1990, 1992; Subramanian & Bhat 1987; Wu & al. 1997).

When the generic concept of *Idriella* was expanded, it became a heterogeneous genus (Seifert & al 2011). In the generic type, *I. lunata*, conidiophores are unbranched and mostly reduced to conidiogenous cells producing unicellular conidia on tiny denticles arranged along the apical and subapical surface of each conidiogenous cell after several sympodial holoblastic extensions (Nelson & Wilhelm 1956), but many other *Idriella* species produce conidiophores branched in various patterns, sympodial extensions of the conidiogenous cells confined to a small, nodose area at the apex, and conidiogenous loci as inconspicuous denticles that produce unicellular or septate conidia. DNA sequences of several *Idriella* species revealed the polyphyletic nature of this genus, showing phylogenetic separation between *I. lunata* (type species) and four other *Idriella* species, which consequently were transferred to four new genera: *Castanediella* Hern.-Restr. & al., *Idriellopsis* Hern.-Restr. & Crous, *Neoidriella* Hern.-Restr. & Crous, and *Paraidriella* Hern.-Restr. & Crous (Crous & al. 2015, Hernández-Restrepo & al. 2016).

During a workshop on fungal diversity conservation in Cacao forest areas, two surveys of microfungi were conducted in *Theobroma cacao* plantations in Guayaquil, Guayas Province, Ecuador, and several *Idriella*-like specimens were found. LSU and ITS sequence analyses place these specimens as incertae sedis

and separate from *Microdochiaceae* (*Xylariales*). They are not congeneric with *Idriella lunata* (the generic type), and we propose a new genus *Guayaquilia* to accommodate them.

Materials & methods

Sampling and fungal strains studied

Samples of decaying plants were placed in plastic bags, transported to the laboratory, placed in moist chambers, and treated according to Castañeda-Ruiz & al. (2016). Individual conidia were separated from the plant material under a stereoscope using an entomological needle and cultured in two different media: V8 (V8: 125 ml V8 juice, 18 g agar, 1000 ml distilled water, pH 6.3) and Cornmeal agar (CMA: 20 g cornmeal, 18 g agar, 1000 ml distilled water, pH 6.3). Morphological observations were made from cultures grown on CMA after five days, incubated at 25 ± 1°C under 12 h alternating near-UV light and darkness (using Vica, FLB-20W T10 near-UV lamp in an irradiation box). Colony colors were coded according to Rayner (1970). Mounts were prepared in lactic acid (90%) or in polyvinyl alcohol-glycerol (8 g PVA in 100 mL of water + 5 mL of glycerol) and lactofuchsin (0.1 g acid fuchsin, 100 mL 85% lactic acid) (Carmichael 1955). Microscopic characters were measured at ×1000 using a Nikon Eclipse Ni-U microscope with DIC optics and a Nikon DS-Fi2 camera.

Pure cultures were deposited at the Microbial Culture Collection of the Biotechnology Research Center, Guayaquil, Ecuador (CCM-CIBE), and the voucher specimens and slide preparations were deposited in the Herbarium, Departamento de Micología, Universidade Federal de Pernambuco, Recife, Brazil (URM). Also examined were the holotype and another specimen deposited as *Idriella cubensis* in the INIFAT Fungus Collection.

DNA extraction, sequencing, and phylogenetic analysis

Genomic DNA was extracted from fresh mycelia grown on V8 Agar at 25°C using a modified rapid extraction method for filamentous fungi according to Cenis (1992). The primer pairs ITS1/ITS4 (White & al. 1990) and LROR/LR5 (Vilgalys & Hester 1990) were used for the amplification of the internal transcribed spacers (ITS), and part of the large subunit of the nuclear ribosomal RNA gene (LSU). PCR cycling conditions followed Korabecna (2007; ITS) and Yang & al. (2017; LSU). PCR products were sent to Macrogen Korea for purification and sequencing with the same primers.

Sequences were aligned and edited using MEGA v.6.0. Phylogenetic relationships among taxa were addressed through Maximum Likelihood (ML) analysis using MEGA v.6.0 and Bayesian analysis using Beast v1.10.4 (Drummond & al. 2012). Data for each gene or region was analyzed both individually and together as a combined data set. jModeltest v.2.1.10 (Darriba & al. 2012) was used to determine the best nucleotide substitution model for both studies. Congruence between individual gene data sets was tested using the partition homogeneity test (Farris & al. 1995) implemented in PAUP v4.0b10 (Swofford, 2001), using a heuristic search option with random taxon addition and TBR branch swapping with 1000 replicates. Tree topologies from ML

analysis of individual genes were also compared visually for congruence. For the combined ML study, GTR was specified as the evolutionary model with estimated proportion of invariables sites and gamma distribution as default parameters. Number of substitution rates was set to four and topology search changed to SPR for tree improvement. Nodal support was assessed by bootstrap analysis from 1000 replicates. Bootstrap values equal or higher than 70% were considered significant. Bayesian inference was run with four Monte Carlo Markov (MCM) chains over 1 million generations with a sampling frequency of 1000 trees using the GTR+I+G as the best model (Drummond & al. 2012). To detect if the sample distribution has reached stationarity, convergence and the effective sample size (ESS) were checked for each run. Posterior probabilities (PP) for the Bayesian analysis were determined by calculating 50% majority rule consensus tree and added onto congruent nodes of the ML tree topology. PP equal or above 0.95 were considered significant. Relevant sequences of ITS and LSU from Hernández-Restrepo & al. (2016) obtained from GenBank, were included in the phylogenetic inference for comparison purposes (Table 1).

Table I. Sequences of *Guayaquilia cubensis* and related species used in the phylogenetic analysis. New sequences are indicated in bold.

Species	Strain #	ITS	LSU	Reference
Astrocystis concavispora	IT1612	KP297404	KP340545	Daranagama & al. 2015
Castanediella acaciae	CBS 139896	KR476728	KR476763	Crous & al. 2015
C. cagnizarii	CBS 542.96	KP859054	KP858991	Hernández-Restrepo & al. 2016
	CBS 101043	KP859051	KP858988	Hernández-Restrepo & al. 2016
C. couratarii	CBS 579.71	KP859050	KP858987	Hernández-Restrepo & al. 2016
Guayaquilia cubensis	MUCL 39017	KC775733	KC775708	Becerra-Hernández & al. 2016
	CCMCIBE-H312	MH777025	MH777024	This Study
	CCMCIBE-H320	MH777026	MH777023	This Study
Idriella lunata	CBS 177.57	KP859043	KP858980	Hernández-Restrepo & al. 2016
	CBS 204.56	KP859044	KP858981	Hernández-Restrepo & al. 2016
	CBS 209.60	KP859045	KP858982	Hernández-Restrepo & al. 2016
	CBS 736.74	KP859046	KP858983	Hernández-Restrepo & al. 2016
Idriellopsis uncinospora	CBS 575.92	KP859052	KP858989	Hernández-Restrepo & al. 2016
Kretzschmaria deusta	CBS 163.93	KC477237	KY610458	Wendt & al. 2018

Microdochium albescens	CBS 243.83	KP858994	KP858930	Hernández-Restrepo & al. 2016
M. citrinidiscum	CBS 109067	KP859003	KP858939	Hernández-Restrepo & al. 2016
M. lycopodinum	CBS 109398	KP859005	KP858941	Hernández-Restrepo & al. 2016
M. majus	CBS 741.79	KP859001	KP858937	Hernández-Restrepo & al. 2016
M. neoqueenslandicum	CBS 445.95	KP858997	KP858933	Hernández-Restrepo & al. 2016
M. nivale	CBS 116205	KP859008	KP858944	Hernández-Restrepo & al. 2016
M. seminicola	CBS 122707	KP859007	KP858943	Hernández-Restrepo & al. 2016
Microdochium sorghi	CBS 691.96	KP859000	KP858936	Hernández-Restrepo & al. 2016
M. tainanense	CBS 269.76	KP859009	KP858945	Hernández-Restrepo & al. 2016
M. trichocladiopsis	CBS 623 77	KP858998	KP858934	Hernández-Restrepo & al. 2016
Neoidriella desertorum	CBS 985.72	KP859048	KP858985	Hernández-Restrepo & al. 2016
Paraidriella jambosae	CBS 374.90	KP859049	KP858986	Hernández-Restrepo & al. 2016
Poronia punctata	CBS:656.78	KT281904	KY610496	Wendt & al. 2018
Rosellinia aquila	MUCL 51703	KY610392	KY610460	Wendt & al. 2018
R. corticium	MUCL 51693	KY610393	KY610461	Wendt & al. 2018
R. necatrix	CBS 349.36	AY909001	KF719204	Peláez & al. 2008
Sarcoxylon compunctum	CBS 359.61	KT281903	KY610462	Wendt & al. 2018
Selenodriella cubensis	CBS 683.96	KP859053	KP858990	Hernández-Restrepo & al. 2016
S. fertilis	CBS 772.83	KP859055	KP858992	Hernández-Restrepo & al. 2016
Xylaria arbuscula	CBS:126415	KY610394	KY610463	Fournier & al. 2011; Wendt & al. 2018
X. hypoxylon	CBS 122620	KY610407	KY610495	Wendt & al. 2018
X.polymorpha	MUCL 49884	KY610408	KY610464	Wendt & al. 2018

Phylogeny

The LSU-ITS dataset comprised 36 aligned sequences with 1482 positions. Fig. 1 presents the ML tree including BS and PP values. The partition homogeneity test showed no significant incongruence between the combined data sets ($p > 0.05$). Trees obtained from ML and Bayesian analysis of the individual loci and the combined analysis produced congruent topologies.

Phylogenetic inferences grouped the three strains identified as *Idriella cubensis* (MUCL 39017, CCMCIBE-H312, CCMCIBE-H320) together in a

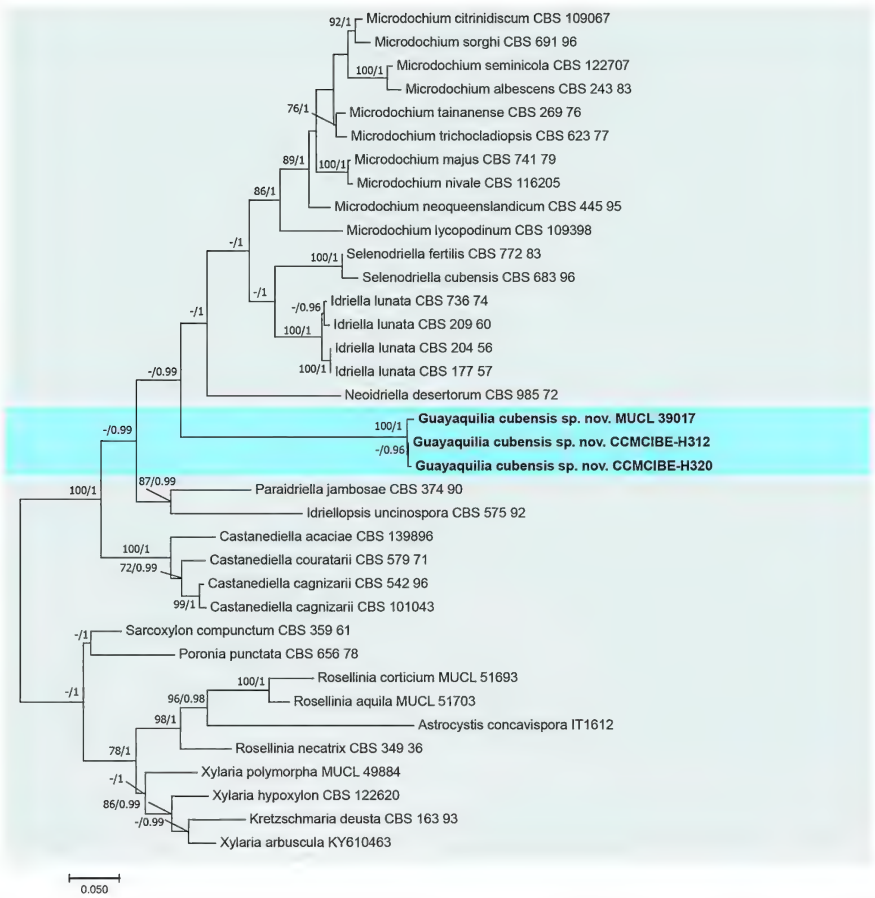

FIG. 1. Maximum Likelihood (ML) non-rooted tree inferred from the combined LSU and ITS sequences of *Guayaquilia cubensis* and related species. Numbers on the branches are support values >75% from BS and >0.95 from PP. The scale bar shows the expected changes per site.

supported clade and sister to *Neoidriella desertorum*, both clades separate from, and basal to, the *Microdochiaceae* Hern.-Restr. & al. clade containing *Idriella lunata*, *Microdochium* spp., and *Selenodriella* spp. We therefore propose a new genus *Guayaquilia* typified by *Idriella cubensis*.

Taxonomy

Guayaquilia R.F. Castañeda, Magdama, D. Sosa & Hern.-Restr., **gen. nov.**

MB 831849

Differs from *Neodriella* and *Paraidriella* by its macronematous, tree-like, irregularly multibranched conidiophores and its intercalary, discrete conidiogenous cells.

Type Species: *Idriella cubensis* R.F. Castañeda & G.R.W. Arnold [≡ *Guayaquilia cubensis* (R.F. Castañeda & G.R.W. Arnold) R.F. Castañeda & al.].

Etymology: *Guayaquilia* (Latin), referring to Guayaquil city, Ecuador.

Asexual. Colonies on natural substrate effuse, pulvinate-velutinous, golden brown to brown. Conidiophores macronematous, mononematous, fasciculate, erect, cylindrical, irregularly branched, septate, smooth, brown. Conidiogenous cells polyblastic, denticulate, sympodial extended, terminal or intercalary, cylindrical or slightly inflated, pale brown to brown. Conidial secession schizolytic. Conidia solitary, acropleurogenous, sublunate, subnavicular, inequilateral, unicellular or septate, hyaline, smooth. Chlamydospores solitary, bicellular, brown and the apical cell globose, thick walled, smooth.

Guayaquilia cubensis (R.F. Castañeda & G.R.W. Arnold) R.F. Castañeda, Magdama, D. Sosa & Hern.-Restr., **comb. nov.** Figs 2, 3

MB 831851

≡ *Idriella cubensis* R. F. Castañeda & G.R.W. Arnold, Rev. Jard. Bot. Nac., Univ. Habana 6(1): 50 (1985).

Colonies on V8 agar at 25°C attaining 60 mm diam after five days, floccose, pale lavender grey to buff, sporulation poor, sparse. On CMA reaching 50 mm diam, felted, dark brick at the center, whitish toward the periphery. Conidiophores macronematous, mononematous, tree-like, fasciculate, profuse, dichotomously, alternately or irregularly branched toward the apex, slightly inflated or bulbous at the base, smooth, brown below, pale brown to subhyaline at the tip of branches, <500 µm long, 4–7.5 µm wide at the base, branches 2–3.5 µm wide. Conidiogenous cells polyblastic, denticulate, intercalary and terminal, in branches integrated, cylindrical, or slightly curved, brown to pale brown or subhyaline, 8–25 × 2–3 µm. Conidia solitary, acropleurogenous, sublunate, subnavicular, lunate, inequilateral, (0–) 1-septate, 14–17 × 2.5–3 µm. Chlamydospores bicellular, the basal cells subcampanulate, subcuneiform to hemispherical, 5–8 µm wide, and the apical cells globose, 18–22 µm diam, brown, thick walled; both cells with lumina granulose, brown; terminal, smooth, arising from assimilative hyphae.

specimens examined: **ECUADOR, Guayas Province, Naranjal**, 2°48′S 79°40′W, on decaying leaves of *Theobroma cacao* L. (*Malvaceae*), 8 July 2017, F. Espinoza & S. Pérez-Martínez (URM 91815 = CCMCIBE-H312; GenBank MH777025, MH777024); 2°41′S 79°36′W, on decaying leaves of *Theobroma cacao*, 8 July 2017, F. Espinoza & S. Pérez-Martínez (URM 91815a = CCMCIBE-H320; GenBank MH777026, MH777023). **CUBA, La Habana Province, Santiago de Las Vegas**, 22°58′N 82°22′W, on decaying leaves of *Calophyllum calaba* L. (*Clusiaceae*), 29 Jun 1983, RF Castañeda Ruiz (holotype,

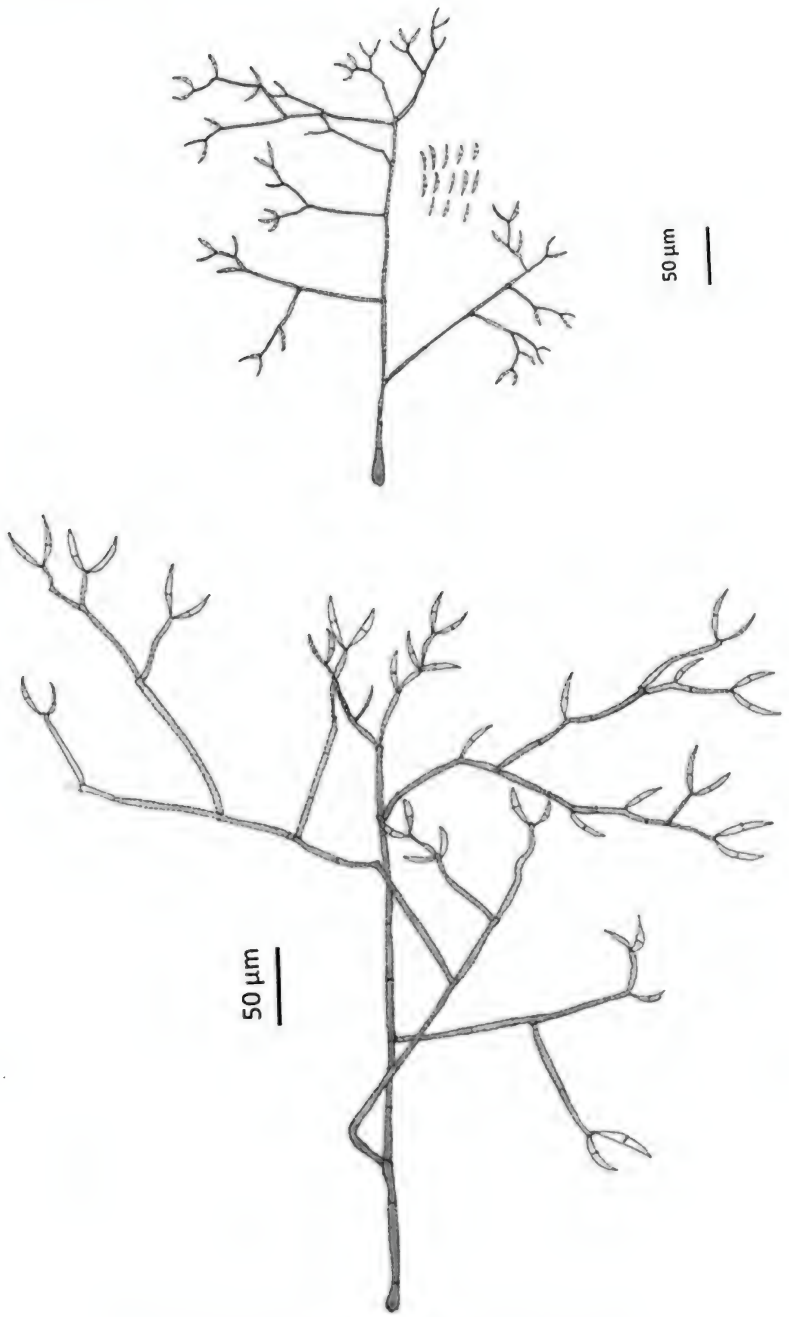

Fig. 2. *Guayaquilia cubensis* (holotype, INIFAT C83/57-1). Conidiophores, conidiogenous cells, and conidia.

Fig. 3. *Guayaquilia cubensis* (CCMCIBE-H312). A. Colony on V8 agar; B. Colony on CMA; C, D. Conidiophores; E–G. Conidiogenous cells; H–L. Conidia; M, N, Chlamydospores. Scale bars = 10 µm.

INIFAT C83/57-1); **Pinar del Rio Province**, **Los Portales**, 22°40′N 83°28′W, on decaying leaves of *Cupania* sp. (*Sapindaceae*), 5 Feb. 1994, RF Castañeda Ruiz, (INIFAT C94/27 = MUCL 39017).

NOTE: *Guayaquilia* shares some morphological characters with the genera *Castanediella*, *Idriella*, *Idriellopsis*, *Neoidriella*, and *Paraidriella*, such as blastic conidial ontogeny and denticulate conidiogenous loci, but the tree-like, dichotomously, alternately or irregularly profuse branched conidiophores with intercalary and terminal conidiogenous cells are present only in *Guayaquilia*. Also, the bicellular, globose, brown chlamydospores that arise from assimilative hyphae in *Guayaquilia* are another distinctive character. The conidiophore morphology in *Guayaquilia* resembles that of some *Phaeodactylium* species (*P. biseptatum*, *P. curvularioides*, and *P. stadleri*) described by Castañeda-Ruiz & al. (2009, 2013) and Matsushima (1980), but these taxa have clavate, ellipsoidal, to obovoid, brown to subhyaline conidia.

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Distoseptispora longispora sp. nov. from freshwater habitats in China

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Abstract—A novel species, *Distoseptispora longispora*, from submerged wood in Yunnan Province, China, is described and illustrated. The fungus is characterized by macronematous, mononematous, septate conidiophores with monoblastic, integrated, determinate, terminal conidiogenous cells that produce acrogenous, solitary, obclavate, elongated, distoseptate conidia. SSU-ITS-LSU sequence analyses were used to infer the phylogenetic relationship between *D. longispora* and related species. Both molecular analyses and morphological data well support *D. longispora* as an independent taxon. A key to *Distoseptispora* species is provided.

Key words—*Ascomycota,* asexual fungi, *Distoseptisporaceae,* hyphomycetes

Introduction

Distoseptispora K.D. Hyde & al. was introduced by Su & al. (2016) to accommodate two species, *D. fluminicola* McKenzie & al., the type species,

and *D. aquatica* Z.L. Luo & al. The genus is characterized by hairy colonies, macronematous conidiophores, monoblastic conidiogenous cells, and distoseptate acrogenous cylindrical conidia., *Distoseptispora* represents a phylogenetically distinct lineage in *Sordariomycetes* for which Su & al. (2016) proposed a new family, *Distoseptisporaceae* K.D. Hyde & McKenzie. Subsequently, 13 more species have been added: *D. cangshanensis* Z.L. Luo & al., *D. dehongensis* W. Dong & al., *D. guttulata* Jing Yang & K.D. Hyde, *D. martinii* (J.L. Crane & Dumont) J.W. Xia & X.G. Zhang, *D. obpyriformis* Z.L. Luo & H.Y. Su, *D. palmarum* S.N. Zhang & al., *D. phangngaensis* Jing Yang & al., *D. rostrata* Z.L. Luo & al., *D. submersa* Z.L. Luo & al., *D. suoluoensis* Jing Yang & al., *D. thailandica* Tibpromma & K.D. Hyde, *D. thysanolaenae* Goonas. & al., and *D. xishuangbannaensis* Tibpromma & K.D. Hyde (Hyde & al. 2019, Luo & al. 2018, Phookamsak & al. 2019, Tibpromma & al 2018, Xia & al. 2017, Yang & al. 2018).

Many aquatic fungi have been discovered in China's freshwater habitats, which are rich in lignicolous fungi (Hu & al. 2007, 2012a,b; Huang & al. 2016; Song & al. 2018). Our ongoing survey of aquatic fungi in Yunnan Province revealed an interesting *Distoseptispora* species that we describe as new based on morphological characters and molecular analyses (SSU-ITS-LSU).

Materials & methods

Sample collection & morphological studies

Debarked wood samples from different freshwater habitats in Yunnan Province, China, were taken to the lab for detection of fungi using dissecting and compound microscopes. The dried specimens were deposited in the Herbarium of Fungi, Jiangxi Agricultural University, Nanchang, China (HFJAU).

DNA extraction, sequencing, and phylogenetic analyses

Genomic DNA was extracted from fresh fungal materials found on the natural substrate following Hu & al. (2012c). We amplified three rDNA regions: internal

Table 1. Isolates included in the phylogenetic analyses.

Species	Strain	GenBank accession numbers		
		ITS	LSU	SSU
Distoseptispora "adscendens"	HKUCC 10820	—	DQ408561	—
D. aquatica	MFLUCC 15-0374[T]	NR_154040	KU376268	—
	SNJ14	**MH555360**	—	—
D. fluminicola	MFLUCC 15-0417[T]	NR_154041	KU376270	—
D. guttulata	MFLUCC 16-0183	MF077543	MF077554	MF077532

D. "leonensis"	HKUCC 10822	—-	DQ408566	—-
D. longispora	**HFJAU 0705^{T}**	**MH555359**	**MH555431**	**MH555357**
D. martinii	HSAUP myr4280	KU999975	KX033566	KX033537
D. multiseptata	MFLUCC 15-0609^{T}	NR_154017	KX710140	MF077540
	MFLUCC 16-1044	MF077544	MF077555	MF077533
D. phangngaensis	MFLUCC 16-0857	MF077545	MF077556	MF077534
D. suoluoensis	MFLUCC 17-1305	MF077547	MF077558	MF077536
	MFLUCC 17-0224	MF077546	MF077557	MF077535
D. tectonae	MFLUCC 12-0291	KX751711	KX751713	—
	GZ25	**MH555361**	—	**MH555358**
D. tectonigena	MFLUCC 12-0292^{T}	NR_154018	KX751714	—
Distoseptispora sp.	HLXM 15-1	—-	KU376269	—-
Dothidea sambuci	AFTOL-ID 274	DQ491505	AY544681	AY544722
Ellisembia bambusicola	HKUCC 3578	—	DQ408562	—
E. calyptrata	HKUCC 10821	—	DQ408564	—
E. minigelatinosa	NN47497	—	DQ408567	—
Ellisembia sp.	HKUCC 10558	—	DQ408565	—
Sporidesmium aquaticivaginatum	MFLUCC 15-0624	KX710147	KX710142	MF077541
S. aquaticum	MFLUCC 15-0420	—	KU376273	—
S. australiense	HKUCC 10833	—	DQ408554	—
S. fluminicola	MFLUCC 15-0346	—	KU376271	—
S. macrurum	HKUCC 2740	—	DQ408555	—
S. obclavatulum	HKUCC 10834	—	DQ408556	—
S. olivaceoconidium	MFLUCC 15-0380	KX710144	KX710139	MF077542
S. pachyanthicola	HKUCC 10835	—	DQ408557	—
S. parvum	HKUCC 10836	—	DQ408558	—
S. pyriformatum	MFLUCC 15-0620^{T}	KX710146	KX710141	—
	MFLUCC 15-0627	KX710148	—	KX710143
S. submersum	MFLUCC 15-0421	—	KU376272	—
S. tengii	HKUCC 10837	—	DQ408559	—
S. thailandense	MFLUCC 15-0964^{T}	MF374361	MF374370	—
S. tropicale	MFLUCC 16-0185	MF077551	MF077562	MF077539
	HKUCC 10838	—	DQ408560	—
Sporidesmium sp.	KUFA 0043	KT824799	—-	—
Sporidesmium sp.	MFLUCC 15-0617	MF077550	MF077561	—-
Sporidesmium sp.	MFLUCC 16-0186	MF077549	MF077560	MF077538

transcribed spacer (ITS) with the primer pair ITS1 & ITS4 (White & al. 1990), partial small subunit (SSU) with NS1 & NS4 (White & al. 1990), and large subunit (LSU) with LR0R & LR6 (Vilgalys & Hester 1990, Rehner & Samuels 1995). PCR protocols followed the conditions set by Hu & al. (2012b). The PCR products were purified and sequenced by the same primers used for PCR at Sangon Biotech (Shanghai) Co. Ltd.

In this study, we generated six novel sequences (MH555360, MH555359, MH555431, MH555357, MH555361 & MH555358) and retrieved 36 reference sequences from GenBank (Table 1) that were aligned using MAFFT v7.

The ML analyses were produced with RAxML v7.2.6 (Stamatakis & Alachiotis 2010) using a GTRGAMMA substitution model with 1000 bootstrap replicates and evaluated by bootstrap support (MLBS).

The best-fit evolutionary models were estimated using MrModeltest V2.2. Posterior probabilities (PP) (Zhaxybayeva & Gogarten 2002) were determined by Markov chain Monte Carlo sampling (BMCMC) in MrBayes 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run 1,500,000 generations, with trees sampled every 100 generations and the first 25% deleted as burn-in. Posterior probabilities were calculated based on the remaining trees. The novel taxonomic descriptions and nomenclature were deposited in MycoBank (http://www.mycobank.org/).

Phylogenetic results

The six new sequences generated in our study included one LSU, two SSU, and three ITS sequences. The phylogenetic tree based on the three-locus analysis (Fig. 1) shows the relationship between the new taxon and other related species. The concatenated aligned dataset comprised 17 isolates from 13 *Distoseptispora* spp., 23 isolates from 21 *Sporidesmium* (or "*Ellisembia*") spp., and one isolate of *Dothidea sambuci* (Pers.) Fr. (*Dothideomycetes*) as an outgroup. The dataset including alignment gaps comprised 2817 characters: 1348 for SSU, 629 for ITS, and 840 for LSU. The combined dataset ML tree (Fig. 1) with bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) indicates several well-supported clades, with the *Distoseptispora longispora* strain forming a well-supported clade (MLBS = 94%, BPP = 0.68) with other *Distoseptispora* species.

Taxonomy

Distoseptispora longispora H.Y. Song & D.M. Hu **sp. nov.** Fig. 2

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Differs from *Distoseptispora aquatica* by its larger and more septate conidia; and from *D. tectonae* and *D. tectonigena* by its smooth and brown to yellowish brown conidia.

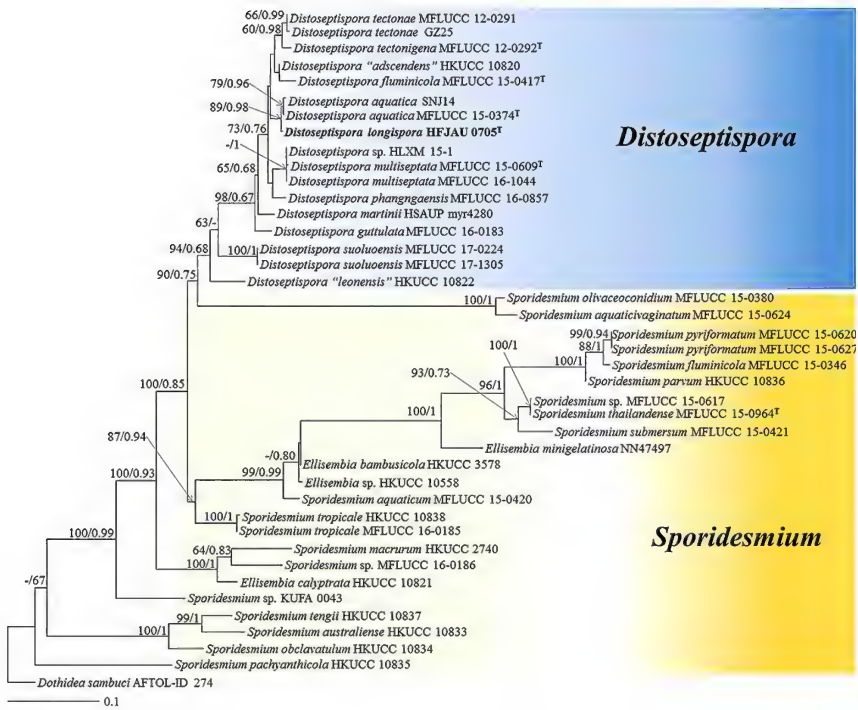
Distoseptispora
Sporidesmium
66/0.99 Distoseptispora tectonae MFLUCC 12-0291
60/0.98 Distoseptispora tectonae GZ25
Distoseptispora tectonigena MFLUCC 12-0292T
Distoseptispora "adscendens" HKUCC 10820
79/0.96 Distoseptispora fluminicola MFLUCC 15-0417T
89/0.98 Distoseptispora aquatica SNJ14
Distoseptispora aquatica MFLUCC 15-0374T
73/0.76 Distoseptispora longispora HFJAU 0705T
-/1 Distoseptispora sp. HLXM 15-1
65/0.68 Distoseptispora multiseptata MFLUCC 15-0609T
Distoseptispora multiseptata MFLUCC 16-1044
98/0.67 Distoseptispora phangngaensis MFLUCC 16-0857
63/- Distoseptispora martinii HSAUP myr4280
Distoseptispora guttulata MFLUCC 16-0183
94/0.68 100/1 Distoseptispora suoluoensis MFLUCC 17-0224
Distoseptispora suoluoensis MFLUCC 17-1305
90/0.75 Distoseptispora "leonensis" HKUCC 10822
100/1 Sporidesmium olivaceoconidium MFLUCC 15-0380
Sporidesmium aquaticivaginatum MFLUCC 15-0624
99/0.94 Sporidesmium pyriformatum MFLUCC 15-0620
100/1 88/1 Sporidesmium pyriformatum MFLUCC 15-0627
100/1 Sporidesmium fluminicola MFLUCC 15-0346
93/0.73 96/1 Sporidesmium parvum HKUCC 10836
100/0.85 Sporidesmium sp. MFLUCC 15-0617
100/1 Sporidesmium thailandense MFLUCC 15-0964T
Sporidesmium submersum MFLUCC 15-0421
87/0.94 Ellisembia minigelatinosa NN47497
-/0.80 Ellisembia bambusicola HKUCC 3578
100/0.93 99/0.99 Ellisembia sp. HKUCC 10558
Sporidesmium aquaticum MFLUCC 15-0420
100/1 Sporidesmium tropicale HKUCC 10838
Sporidesmium tropicale MFLUCC 16-0185
100/0.99 64/0.83 Sporidesmium macrurum HKUCC 2740
100/1 Sporidesmium sp. MFLUCC 16-0186
-/67 Ellisembia calyptrata HKUCC 10821
Sporidesmium sp. KUFA 0043
99/1 Sporidesmium tengii HKUCC 10837
100/1 Sporidesmium australiense HKUCC 10833
Sporidesmium obclavatulum HKUCC 10834
Sporidesmium pachyanthicola HKUCC 10835
Dothidea sambuci AFTOL-ID 274
0.1

FIG. 1. Maximum Likelihood (ML) phylogenetic tree of *Distoseptispora longispora* and allied species based on concatenated SSU–ITS–LSU data. The RAxML bootstrap support values (MLBS) and Bayesian posterior probabilities (BPP) are given at the nodes (MLBS/BPP).

TYPE: China, Yunnan Province: Mengla, a small stream, on submerged wood, 13 Apr 2017, H.Y. Song (**Holotype**, HFJAU 0705).

ETYMOLOGY: *longispora*, referring to the long conidia of the fungus.

COLONIES on submerged wood superficial, effuse, scattered, hairy, brown to dark brown. Mycelium mostly immersed, composed of branched, septate, smooth, subhyaline to pale brown hyphae. CONIDIOPHORES macronematous, mononematous, 2–4-septate, unbranched, single, straight or slightly flexuous, erect, cylindrical, smooth, brown to dark brown, 17–37 × 6–10 µm. CONIDIOGENOUS CELLS monoblastic, integrated, determinate, terminal, light brown to dark brown, cylindrical. Conidial secession schizolytic. CONIDIA acrogenous, solitary, dry, obclavate, elongated, straight or slightly curved, rounded at the apex, truncate at the base, 31–56-distoseptate, smooth, brown

to yellowish brown, slightly paler towards the apex, 189–297 × 16–23 µm (mean = 204 × 21 µm, n = 50).

Additional specimen examined: **CHINA**, **Yunnan Province**, **Mengla**, a small stream, on submerged wood, 13 Apr 2017, H.Y. Song (HFJAU 0706).

Notes: *Distoseptispora longispora* is characterized by its macronematous, mononematous, septate conidiophores, monoblastic, integrated, determinate, terminal conidiogenous cells, and acrogenous, solitary, dry, obclavate, elongated, distoseptate conidia, which fit well with the generic concepts of *Distoseptispora* (Su & al. 2016). The molecular phylogeny (Fig. 1) clusters the *D. longispora* strain well within the *Distoseptispora* clade and closely related to *D. aquatica* but with an ITS six base pair (bp) difference separating it from *D. aquatica* (MFLUCC 15-0374). *Distoseptispora longispora* resembles *D. aquatica*, *D. tectonae,* and *D. tectonigena* in conidial shape. However, its larger and more septate conidia separate *D. longispora* from *D. aquatica* (189–297 × 16–23 µm; 15–28 septa). In addition, *D. tectonae* has verruculose, dark reddish conidia, and *D. tectonigena* has dark reddish-brown conidia (Hyde & al. 2016).

The multilocus molecular phylogeny and morphological data fully support separation of our new species, *D. longispora*. Morphologically, *Distoseptispora* resembles *Ellisembia* Subram., first established to accommodate the species with distoseptate conidia in *Sporidesmium* Link (Subramanian 1992). However, a molecular phylogeny by Shenoy & al. (2006) showed that *Ellisembia* and *Sporidesmium* were polyphyletic. Su & al. (2016) later clarified differences between *Ellisembia* and *Sporidesmium,* noting that euseptate/distoseptate characters were not supported by molecular evidence and treating *Ellisembia* as a synonym of *Sporidesmium*. *Distoseptispora* species differ from *Ellisembia* (*Sporidesmium*) species in having relatively short conidiophores and darker conidia with slightly paler, but not hyaline, rounded apices, basal cells cut off by cross walls, and of indeterminate length (Su & al. 2016).

There are various hyphomycetes in freshwater habitats, and several studies have reported *Distoseptispora* species, e.g., *D. aquatica*, *D. fluminicola*, *D. guttulata*, *D. multiseptata*, *D. phangngaensis,* and *D. suoluoensis* (Hu & al. 2007, 2010a,b; Huang & al. 2018; Hyde & al. 2016; Su & al. 2016; Yang & al. 2018). Most of these species were identified based on morphology, which proved to be insufficient for accurate identification (Hu & al. 2017). Numerous other studies (Réblová & al. 2016, Su & al. 2016, Zhang & al. 2017) have revealed many different freshwater lineages using molecular analyses.

Fig. 2. *Distoseptispora longispora* (holotype, HFJAU 0705). a. Colonies on submerged wood; b, c. Conidia; d. Conidiophore. Scale bars: a = 100 μm, b–d = 5 μm.

Key to *Distoseptispora* species

1. Conidia muriform, having both longitudinal and transverse septa ..... *D. martinii*
1. Conidia with only transverse septa 2
2. Conidia euseptate 3
2. Conidia distoseptate 4
3. Conidia 75–130 × 7–11 µm, 11–14(–20)-euseptate, smooth ..... *D. guttulata*
3. Conidia 80–125 × 8–13 µm, 8–10-euseptate, verrucose ..... *D. suoluoensis*
4. Conidiogenous cells polyblastic, conidia 35–180 × 7–11 µm ..... *D. palmarum*
4. Conidiogenous cells monoblastic 5
5. Conidiophores having percurrently proliferating conidiogenous cells at the apex of conidiophores 6
5. Conidiophores not as above 7
6. Conidia 115–155 × 9–11 µm ..... *D. rostrata*
6. Conidia 148–225 × 11–12 µm ..... *D. tectonigena*
7. Conidia verruculose, 7.5–9.5 × 3.5–5 µm ..... *D. tectonae*
7. Conidia not verruculose 8
8. Conidia 3–5-distoseptate, 17–30 × 7.5–10 µm ..... *D. dehongensis*
8. Conidia with more than 6 distosepta 9
9. Saprobic on submerged wood in freshwater habitats 12
9. Not saprobic on submerged wood in freshwater habitats 10
10. Saprobic on dead culms of *Thysanolaena maxima*, conidia 8–14-distoseptate, 21.5–80 × 6.5–12.8 µm ..... *D. thysanolaenae*
10. Saprobic on dead leaves of *Pandanus* sp. 11
11. Conidia reddish brown to brown, 130–230 × 13.5–17 µm ..... *D. thailandica*
11. Conidia green-brown to brown, 160–305 × 8–15 µm ..... *D. xishuangbannaensis*
12. Conidia 9–11-distoseptate, 53–71 × 12–16 µm ..... *D. obpyriformis*
12. Conidia with more than 12 distosepta 13
13. Conidiogenous cells yellow, conidia 125–250 × 13–15 µm ..... *D. fluminicola*
13. Conidiogenous cells brownish 14
14. Conidia brown to yellowish brown, 31–56-distoseptate, 189–297 × 16–23 µm ..... *D. longispora*
14. Conidia greenish brown or dark olivaceous green 15
15. Conidiogenous cells subhyaline to pale brown, conidia 58–166 × 10–14 µm ..... *D. cangshanensis*
15. Conidiogenous cells brown to dark brown 16
16. Conidiogenous cells dark brown to olivaceous, conidia 110–157 × 13.5–16.5 µm ..... *D. aquatica*
16. Conidiogenous cells brown 17

17. Conidia dark olivaceous green, multi-distoseptate, 95–290 × 11–20 μm ..... *D. multiseptata*
17. Conidia dark or mid olivaceous to mid or dark brown 18
18. Conidia dark olivaceous to mid or dark brown, 165–350 × 14–19 μm ..... *D. phangngaensis*
18. Conidia mid-olivaceous to brown, 95–123 ×15–19 μm ..... *D. submersa*

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Notes on rust fungi in China 9. *Puccinia miscanthi* life cycle and morphology confirmed by inoculation

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Abstract—The Heteroecious and macrocyclic life cycle of *Puccinia miscanthi* is confirmed for the first time with inoculation experiments in China. The rust produces spermogonial and aecial stages on *Plantago asiatica* and uredinial and telial stages on *Miscanthus sacchariflorus.* Morphological characters of these stages are described based on field collections and specimens that confirmed their life cycle connections. The neotype of *P. miscanthi* is designated.

Key words—*Plantaginaceae,* plant disease, *Poaceae, Pucciniales, Uredinales*

Introduction

Puccinia miscanthi was first described with uredinial and telial stages on *Miscanthus sacchariflorus* (*Poaceae*) collected in northeastern China (Miura 1928). The life cycle of *P. miscanthi* was reported in Japan by Hiratsuka (1933), Ito (1934), and Asuyama (1936) under the name of *P. eulaliae* Barclay. These authors demonstrated life cycle connection between spermogonial and aecial stages on *Plantago asiatica* (*Plantaginaceae*) and uredinial and telial stages on *M. sinensis* Andersson through inoculations. Ito (1950) considered the rust fungus as producing spermogonial and aecial stages on *Plantago* and uredinial and telial stages on *Miscanthus* and *Imperata* (as *P. eulaliae*) and treated *P. miscanthi* as a synonym of this species. Later, however, this rust fungus

was considered to represent a species different from *P. eulaliae* because of morphology and host relations, and it was treated as *P. miscanthi* (Cummins 1971, Hiratsuka & al. 1992). Sato & Kakishima (1982) reported *Lysimachia clethroides* Duby (*Primulaceae*) as an additional host of the rust's spermogonial and aecial stages. This rust fungus has been reported on wide range of host plants from northeastern Asia (Table 1, p. 532).

During investigation of rust fungi in northeastern China, a *Puccinia* species producing uredinial and telial stages on *M. sacchariflorus* was collected in Heilongjiang Province. Spermogonial and aecial stages of a rust fungus on *Plantago asiatica* were also found near infected *M. sacchariflorus* plants. The spermogonial and aecial stages were suspected to represent different stages of *Puccinia miscanthi.* However, life cycle connection of *P. miscanthi* between different host plants has not been clarified in China, although *Pl. asiatica, Pl. depressa* Willd., and *Pl. major* L. have all been listed as spermogonial and aecial host plants, and *M. floridulus* (Labill.) K. Schum. & Lauterb., *M. sacchariflorus, M. sinensis, Saccharum spontaneum* L., and *Thysanolaena maxima* (Roxb.) Kuntze as uredinial and telial hosts (Wang & Zhuang 1998, Cao & Li 1999). Furthermore, *Puccinia cynodontis* Lacroix ex Desm. also has been reported to produce spermogonia and aecia on *Plantago* species including *Pl. asiatica* (Cummins 1971, Wang & Zhuang 1998). Worldwide, uredinial and telial stages of six morphologically similar *Puccinia* species have been recorded on *Miscanthus* (Table 1; Cummins 1971, Hiratsuka & al. 1992, Wang & Zhuang 1998). Among them, *P. miscanthi, P. miscanthicola* F.L. Tai & C.C. Cheo, *P. melanocephala* Syd. & P. Syd., and *P. erythropus* Dietel have been recorded in China (Tai 1979, Wang & Zhuang 1998). Therefore, we carried out inoculation experiments to clarify the life cycle connection between spermogonia and aecia on *Pl. asiatica* and uredinia and telia on *M. sacchariflorus* collected in Heilongjiang Province on 2 July 2018. We also observed morphology of these specimens for identification of species.

Materials & methods

Inoculation experiments

On 2 July 2018 uredinia and telia on *Miscanthus sacchariflorus* were found at Wuchang, Heilongjiang Province, China (44°12′22″N 128°03′33″E). Infected plants were transplanted in plastic pots and placed on the ground at the campus of Jilin Agricultural University, Changchun, Jilin Province. In late summer, abundant telia appeared on the plants, and these telia were overwintered on dead leaves. Teliospores obtained from the telia were observed to germinate and basidiospores produced in the spring of 2019 were used for inoculations. On 17 June 2019 small pieces of leaves

Fig. 1. *Puccinia miscanthi* on *Plantago asiatica* (A, C, E) and *Miscanthus sacchariflorus* (B, D, F). A. The plants in plastic pots, producing spermogonia and aecia by basidiospore inoculation. B. Plant transplanted from Heilongjiang Province and cultured at campus of Jilin Agricultural University. C. Spermogonia produced on leaf surface by basidiospore inoculation. D. Brown uredinia produced on leaf surface by aeciospore inoculation. E. Aecia on lower leaf surface produced by basidiospore inoculation. F. Dark brown telia on leaf.

with teliospores were attached to the surfaces of young healthy leaves of *Pl. asiatica* for inoculation, using a similar method previously reported by Ji & al. (2017a,b). The inoculated plants were collected at campus of Jilin Agricultural University and had been maintained in plastic pots. No natural infection of the field plants had

been confirmed prior to inoculation. The inoculated plants were kept in a humid environment in a plastic box in darkness at 20–23 °C for 3 days and then transferred to a place near windows at 20–23 °C for observation.

Aeciospores obtained from aecia on the basidiospore-inoculated *Plantago asiatica* leaves were dusted onto small pieces of wet filter paper (about 3 mm^2) in a Petri dish. These papers were then placed on young healthy leaves of *M. sacchariflorus,* which had been transplanted from Wuchang, Heilongjiang Province in 2018 and maintained in plastic pots at campus of Jilin Agricultural University. Plants with new leaves were used for the inoculation. The inoculated plants were kept in a humid environment in a plastic box under the same conditions as the basidiospore inoculations, and later transferred to the place near windows.

Morphological observations

Specimens collected at Wuchang, and specimens obtained by inoculations were morphologically examined. The spores and size and shape of sori were microscopically examined using Light (LM) and scanning electron (SEM) microscopes according to Ji & al. (2019). The specimens are deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, China (HMJAU).

Results & discussion

Life cycle

About 10 days after inoculation of *Plantago asiatica* with basidiospores from germinating teliospores, small yellow spots of spermogonia appeared on both surfaces of the inoculated leaves (Figs 1A, C). These spots were frequently observed on the leaf, and the leaf tissue around infected veins was sometimes malformed when many spots were produced. About 10 to 12 days later, orange-yellow aecia were produced around the spots on lower surfaces of the leaves (Fig. 1E).

About 5 days after aeciospore inoculation, pale yellow spots appeared on the leaves of *Miscanthus. sacchariflorus*, after which then brown uredinia were produced (Fig. 1D). One to two months after that, blackish telia appeared on *M. sacchariflorus* (Fig. 1F). The inoculation results confirmed that the rust fungus producing spermogonial and aecial stages on *Pl. asiatica* was conspecific with the rust fungus producing uredinial and telial stages on *M. sacchariflorus.*

Morphology & identification

The inoculations show that this rust fungus has an heteromacrocyclic life cycle, producing spermogonia and aecia on *Plantago asiatica* and uredinia and telia on *Micanthus sacchariflorus.* The uredinial and telial stages of our Heilongjiang Province rust on *M. sacchariflorus* are morphologically similar to those of

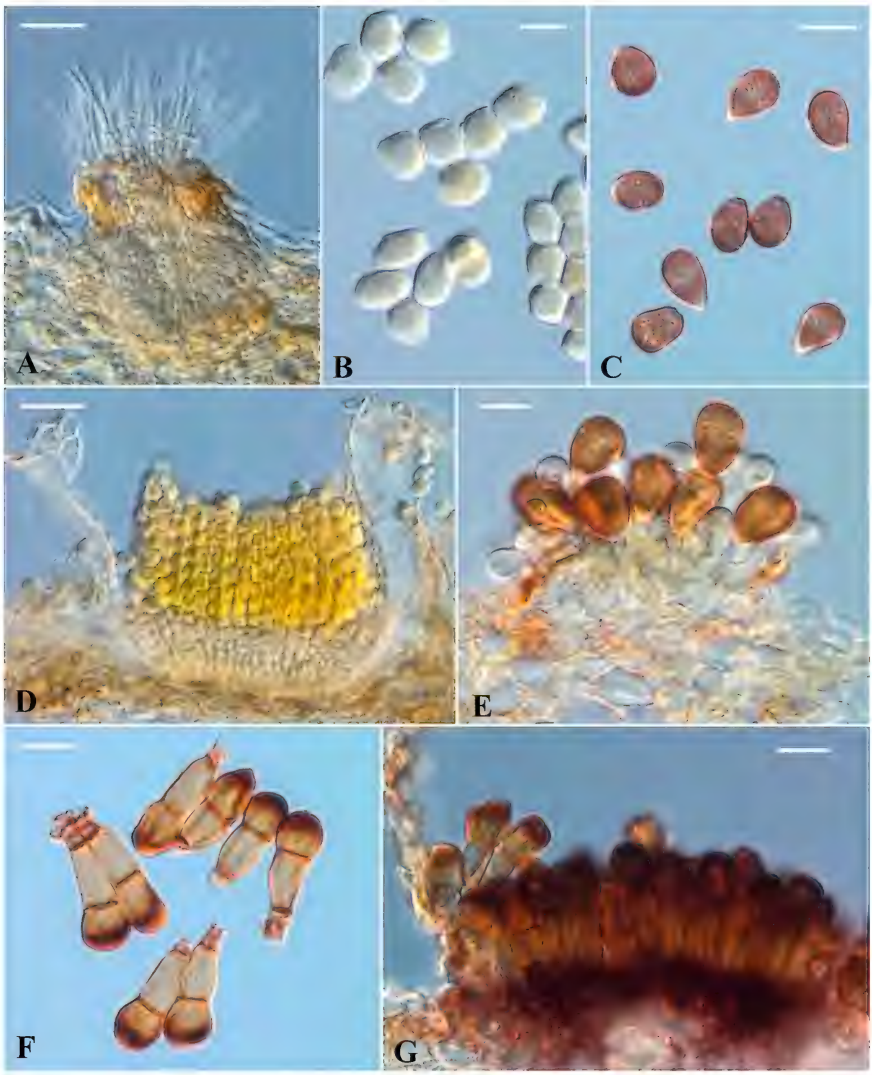

FIG. 2. *Puccinia miscanthi* on *Plantago asiatica* (HMJAU8642: A, B, D) and *Miscanthus sacchariflorus* (neotype, HMJAU8643: C, E, F, G) observed by LM. A. Vertical section of spermogonium (type 4). B. Aeciospores with verrucose surface. C. Urediniospores with equatorial germ pores. D. Vertical section of aecium with catenulate aeciospores surrounded by peridium. E. Vertical section of uredinium mixed with paraphyses. F. Teliospores. G. Vertical section of telia, surrounded by host epidermis. Scale bars: A, C = 30 µm; B, E = 20 µm; D = 100 µm; F, G = 25 µm.

Puccinia erythropus, *P. melanocephala*, and *P. miscanthi* in urediniospore and teliospore shapes. However, teliospores of *P. erythropus* and *P. melanocephala*

are shorter, and their apical walls thinner, than those of our *M. sacchariflorus* rust fungus (Table 1). Furthermore, although Ito (1950) described paraphyses (Table 1), *P. erythropus* has been reported to have no paraphysis in uredinia, whereas, our *M. sacchariflorus* rust fungus produces abundant paraphyses (Figs 2E, 3D). The morphological characters of our *M. sacchariflorus* rust fungus are very close to those previously reported for *P. miscanthi*, but as its teliospores are only slightly shorter than these descriptions (Table 1), we identified our rust fungus as *P. miscanthi.* Host range and morphological features of *P. miscanthi* reported by different authors as shown in Table 1; the differences may be considered variations within species caused by host plants and geographical distribution. *Puccinia miscanthi* has been reported to be genetically very close to *P. melanocephala* although they are morphologically different (Virtudazo & al. 2001, Dixon & al. 2010). Phylogenetic analyses with specimens from different regions and host plants may clarify their relationships.

Spermogonial and aecial stages of *Puccinia miscanthi* have been reported to be produced on *Plantago* and *Lysimachia* species (Cummins 1971, Hiratsuka & al. 1992, Wang & Zhuang 1998). However, no confirmation of these stages of *P. miscanthi* has been reported in China, although its life cycle connection was reported in Japan with inoculations. Therefore, *Pl. asiatica* is the first host plant confirmed by inoculations of its spermogonial and aecial stages in China. The morphology of these stages is almost identical with the descriptions reported from Japan by Ito (1950), Sato & Kakishima (1982), and Hiratsuka & al. (1992), except for the slightly smaller aeciospores. Wang & Zhuang (1998) described the rust's spermogonial and aecial stages on *Pl. depressa* in China; however, a connection to its uredinial and telial stages was not confirmed. Two *Plantago* species, *Pl. asiatica* and *Pl. lanceolata* L., were also reported as spermogonial and aecial host plants of *P. cynodontis* without confirmation of their life cycle connections in China (Wang & Zhuang 1998). Therefore, rust species identifications of spermogonial and aecial stages on species of *Plantago* should be reconsidered. Ono & Azbukina (1997) reported that *P. erythropus*, morphologically similar to *P. miscanthi*, produced spermogonia and aecia on *Cynanchum sublanceolatum* var. *obtusum* (Franch. & Sav.) Matsum. (*Apocynaceae*), suggesting that this species differs from *P. miscanthi* in its spermogonial and aecial hosts.

Miura (1928) described *Puccinia miscanthi* based on a specimen on *Miscanthus sacchariflorus* collected in Teikaton, Manchuria (northeast China) on 22 September in 1919. However, this specimen has not been found in any herbarium, we therefore designate a neotype. We collected specimens on the

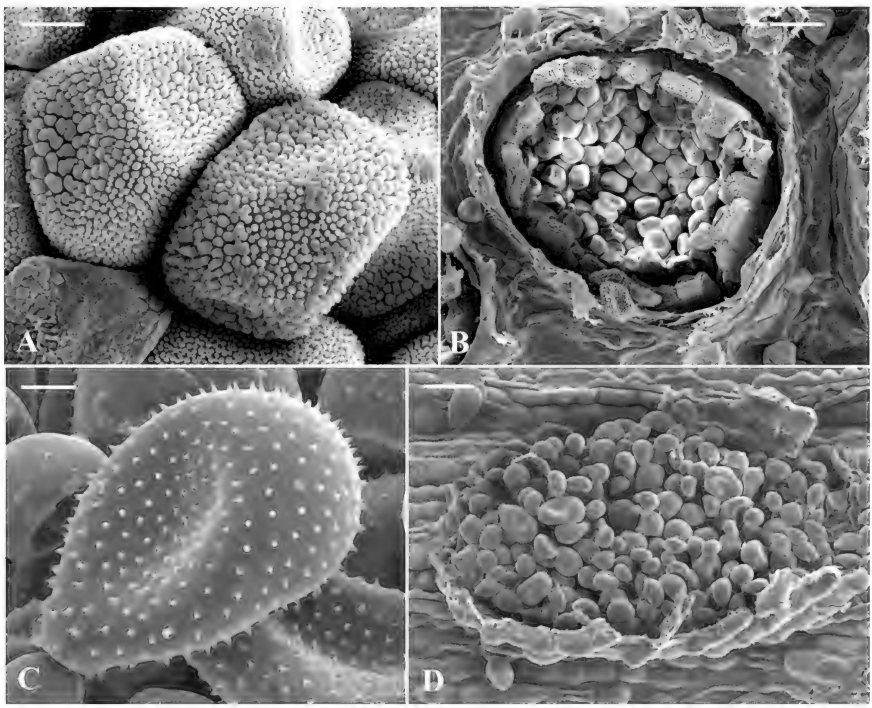

Fig. 3. *Puccinia miscanthi* on *Plantago asiatica* (HMJAU8642: A, B) and *Miscanthus sacchariflorus* (neotype, HMJAU8643: C, D) observed by SEM. A. Aeciospores with densely verrucose surface. B. Aecium with peridium. C. Urediniospore with echinulate surface. D. Uredinium mixed with paraphyses. Scale bars: A, C = 5 μm; B = 40 μm; D = 30 μm.

same host plant from the same area of China, as reported by Miura (1928) and used for inoculations. We believe that specimens collected and obtained from inoculations are morphologically close to the original specimen and describe its morphology based on these specimens.

Puccinia miscanthi Miura,

Fl. Manch. E. Mong., III Cryptogams, Fungi: 302, 1928. Figs 1–3

Type: Uredinia and telia on *Miscanthus sacchariflorus* (Maxim.) Franch. (*Poaceae*), China, Helongjiang Province, Wuchang, 9 September 2017, leg. M. Kakishima & J.X. Ji (**neotype designated here,** HMJAU8643; MBT388544).

Spermogonia amphigenous, surrounded by greenish yellow lesions, densely grouped, yellow, subepidermal, flask-shaped, type 4 of Cummins & Hiratsuka (2003). Aecia hypophyllous, densely grouped, yellow, cupulate with peridia, *Aecidium*-type. Aeciospores catenulate, globose to subglobose,

Table 1. Comparative morphology of uredinia and telia of *Puccinia* species reported on *Miscanthus* spp.

Species	Host genera[1]	Distribution	Paraphyses in uredinium[2]	Urediniospores		Teliospores			Reference[3]
				Size (µm)	Pore no.	Size (µm)	Wall thickness (µm)		
							Side	Apex	
P. miscanthi [= *P. eulaliae* S. Ito, non Barclay]	M	China, Taiwan, Japan, Philippines, Russia	+	27–35.5 × 19.5–23.5	4	39.5–56.5 × 14–22	0.5–1	2.6–6	The present paper
	M		?	33 × 25	10	43–61 × 21–25	?	7–8.5	Miura 1928*
	I, M		+	25–38 × 20–31	4–5	32–76 × 16–24	?	2–7	Ito 1950
	I, M, S		+	29–35 × 19–26	4–5	40–60 × 16–23	1.5–2	4–6	Cummins 1971
	I, M, P		+	29–35 × 19–26	4–5	40–60 × 16–23	1.5–2	4–6	Hiratsuka & al. 1992
	T, M, S		+	25–38 × 20–27	4–5	32–76 × 16–24	1.5–2	2–8	Wang & Zhuang 1998
	M		+	27–38 × 20–32	4–5	32–60 × 16–24	1.5–2	4–6	Azbukina 2005
P. miscanthicola	M	China		Uredinia unknown		32–55 × 15–24	2–3	2–3	Cummins 1971
	M					32–55 × 15–24	2–3	2–4	Wang & Zhuang 1998
P. miscanthidii	M	South Africa	–	24–30 × 19–23	3–4	33–46 × 20–27	2–3	5–9	Cummins 1971
P. melanocephala	?	Worldwide	+	22–40 × 18–25	?	35–45 × 14–20	?	4–6	Sydow & Sydow 1907*
	E, S		+	28–33 × 18–23	4–5	30–43 × 17–21	1.5–2	3–4	Cummins 1971
	S		+	28–33 × 18–23	4–5	30–43 × 17–21	1.5–2	3–4	Hiratsuka & al. 1992
	E, S, M		+	27–34 × 18–24	4–5	30–43× 17–22	1.5–2	3–4	Wang & Zhuang 1998
P. daisenensis	M	Japan, Russia	+	28–34 × 21–28	3–5	42–60 × 15–21	?	9–13.5	Ito 1950
	M		+	26–33 × 19–23	4–5	35–56 × 15–22	1.5–2	7–13	Cummins 1971
	M		+	26–33 × 19–23	4	35–56 × 15–22	1.5–2	7–13	Hiratsuka & al. 1992
P. erythropus	M	China, Taiwan, Japan, Philippines, Russia	?	23–28 × 18–23	4	28–45 × 16–21	?	4	Dietel 1905*
	M		+	28–34 × 20–25	4	32–52 × 14–22	2–3		Ito 1950
	E, M		–	24–33 × 18–23	3–4	33–45 × 16–20	1.5–2.5	3–5	Cummins 1971
	M		–	24–33 × 18–23	3–4	33–45 × 16–20	1.5–2.5	3–5	Hiratsuka & al. 1992
	S, M		–	24–32 × 18–23	3–4	32–45 × 17–21	1.5–2.5	3–5	Wang & Zhuang 1998

1) E: *Erianthus*; I: *Imperata*; M: *Miscanthus*; S: *Saccharum*; T: *Thysanolaena*; P: *Phacelurus.* 2) +: Presence; –: Absence; ?: Not given. 3) *: Original description

angular, 17.5–23.5 × 14.5–19.0 µm (av. = 20.5 × 17. 0 µm); walls hyaline, 0.6–1.2 µm (av.= 0.9 µm) thick, densely verrucose.

Uredinia mostly on abaxial surface, scattered or gregarious, yellow-brown, subepidermal, erumpent, with abundant paraphyses. Paraphyses capitate; walls hyaline, thicker at the apical parts. Urediniospores pedicellate, obovoid to ellipsoid, 27.0–35.5 × 19.5–23.5 µm (av. = 30.0 × 21.5 µm); walls brown, echinulate, 0.7–1.5 µm (av. = 1.0 µm) thick; germ pores mostly 4, equatorial. Telia mostly on abaxial surface, blackish brown, erumpent, pulvinate. Teliospores pedicellate, oblong-clavate, 39.5–56.5 × 14.0–22.0 µm (av. = 47.0 × 19.0 µm); walls chestnut-brown, 0.5–1.5 µm (av. = 1.0 µm) thick at sides, 2.5–6.0 µm (av. 4.0 µm) thick at apices, smooth; pedicels persistent, brown, thick-walled, short.

Additional specimens examined — **CHINA, Heilongjiang Province, Wuhan**: Spermogonia and aecia on *Plantago asiatica*, 2 July 2018, (HMJAU8644); cultured at Jilin Agricultural University, Changchun, 5 July 2019 (HMJAU8642). Uredinia and telia on *Miscanthus sacchariflorus*, 2 July 2018 (HMJAU8645); cultured at Jilin Agricultural University, Changchun, 5 July 2019 (HMJAU8646).

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Biodiversity of heat-resistant ascomycetes from semi-arid soils in Argentina

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Abstract—Artificial thermal shock conducted on 50 soil samples from a semi-arid geographic region (Catamarca and La Rioja provinces) in northern Argentina yielded 34 heat-resistant fungal strains. These strains were assigned to seventeen taxa in ten ascomycete genera: *Arthrinium* (1), *Aspergillus* (3), *Epicoccum* (1), *Gilmaniella* (1), *Hamigera* (2), *Leiothecium* (1), *Penicillium* (2), *Talaromyces* (4), *Trichocladium* (1), and *Trichoderma* (1). All strains were identified by phenotypic features, with molecular data additionally obtained for eleven strains. Five species are reported for the first time in Argentina.

Key words —*Ascomycota*, *Eurotiales*, South America, Monte, Chaco.

Introduction

Temperature is an environmental parameter that plays a key role in the survival, growth, distribution, and speciation of microorganisms on earth (Mouchacca 1993). Fungi growing at high temperatures are generally classified

in two groups: thermophilic and thermotolerant. Thermophilic fungi are those able to grow in a temperature range of 20–50 °C or above; while the maximum temperature for growth of thermotolerant fungi is near 50 °C with a minimum of below 20 °C (Cooney & Emerson 1964, Mouchacca 2007).

Alternatively, heat-resistant fungi are defined as those capable of surviving to a thermal shock, e.g., temperatures at or above 75 °C for 30 minutes or more (Samson & al. 2000). The fungal structures able to survive these extreme temperatures are mainly ascospores, but other propagules such as chlamydospores, thick-walled hyphae, and sclerotia, also may survive such temperature extreme (Scholte & al. 2004). Dijksterhuis & Samson (2006) expanded the concept of heat-resistant fungi to include most of fungal structures that survive after a thermal shock between 55–95 °C. Heat resistance is associated with the ability to maintain the viability of certain fungal structures in order to overcome natural thermal shock. Thus, heat-resistant fungi can be mesophilic, thermotolerant, or thermophilic.

Soil, the primary reservoir of fungi, is also the main repository of dormant propagules of true soil-borne fungi as well as of fungi that grow elsewhere. Most fungi achieve survival through the production of both dormant and dispersal spores (Carlile & Watkinson 1994). Latent spores normally germinate due to slow dormancy decay or in response to a specific (physical or chemical) stimulus that breaks the dormancy (Carlile & Watkinson 1994), such as a thermal shock. Constitutive dormancy is a condition in which the development is delayed by an innate property, such as a barrier to the penetration of nutrients (including water), a metabolic blockage, or the action of a self-inhibitory molecule (Dijksterhuis & Samson 2006, Dijksterhuis 2007). Ascospores, the sexual propagules of the *Ascomycota,* often have a long survival capability (Dijksterhuis 2007). Ascospores of heat-resistant fungi exhibit constitutive dormancy and require a robust signal (heat, high pressure, or exposure to organic compounds, such as dimethylketone) to break the dormancy (Dijksterhuis 2007, Stchigel 2000). Warcup & Baker (1963) noted an increase in the number of ascomycete colonies when soil samples were exposed to temperatures between 50–75 °C for 30 minutes before culturing, compared with those not exposed to this thermal shock.

The fungal soil communities from arid regions are mainly characterized by a low population coupled with a high biodiversity (Mouchacca 1993). During broad research on heat resistant fungi from the semi-arid region of Northern Argentina 194 isolates were obtained. Most isolates were related to *Aspergillus* sect. *Fumigati,* but 34 isolates represented different

interesting taxa. This study documents those 34 miscellaneous heat-resistant ascomycetes isolated from soil.

Materials & methods

Description of the sampling area

Fifty 200-g soil samples were collected in the summer of 2009 and winter of 2011 from several sites in Catamarca and La Rioja provinces, Northern Argentina. Of the six phytogeographical regions of Catamarca (Morláns 1995), most samples were collected from the "monte" region and only a few from the "chaqueña" region; additionally three collections were sampled from the "monte" region of La Rioja (with five phytogeographical regions). The monte region in Catamarca, which comprises c. 14,600 km^2 (Manghi & al. 2005) and extends through the center of La Rioja province, is characterized by steppe vegetation dominated by xerophilous, psammophilous, or halophilous shrubs, and characterized by the almost constant presence of *Larrea* (*Zygophyllaceae*) and *Prosopis* (*Leguminosae*). The climax community is *Larrea divaricata* Cav. ("jarillal"), which develops in the sandy pockets and plains or stony-sandy soils (Cabrera 1971). Climate in the monte is subtropical, arid, with an annual 150–200 mm precipitation occurring mostly in the summer (60–70% of total rainfall; Morláns 1995). Temperature shows a relatively broad seasonal and daily variation, with autumn/winter lows (–30 °C in west mountain region) and spring/summer highs (45 °C in the east and central regions) (Gobierno de Catamarca 2018, Morláns 1995). The strong winds throughout the year favor environmental dryness. The monte elevation ranges from 600–3000 m a.s.l. (Morláns 1995).

Fungal isolation

From each sample, c. 5 g of soil was placed into a conical flask, mixed with 100 ml of sterile melted malt extract agar (Oxoid MEA: malt extract 30.0 g, mycological peptone 5.0 g, agar 15.0 g) plus 50 ppm of chloramphenicol, and heated at 75 °C for 30 min. The suspension was plated into sterile Petri dishes and, once gelled, incubated at 30 °C ≤30 d (Samson & al. 2000). All colonies were transferred to individual Petri dishes containing MEA to obtain pure cultures. The fungal strains were deposited in the culture collection of the Herbarium, Universidad de Buenos Aires, Argentina (BAFC) and the culture collection of Faculty of Medicine, Universitat Rovira i Virgili, Reus, Catalonia, Spain (FMR). Author abbreviations include SMR (S.M. Romero), AIR (A.I. Romero), AMS (Stchigel), ERA (Rodríguez Andrade), VB (Barrera), and RC (Comerio). Abbreviations under Culture(s) examined are RN (Ruta Nacional) and RP (Ruta Provincial).

Morphological study

Preliminary characterization at the genus level was conducted on MEA (Domsch & al. 2007, Guarro & al. 2012). Species were identified by growing the fungal strains on different culture media and temperatures (following the bibliography for each genus mentioned in each description). Vegetative and reproductive structures were

microscopically examined by mounting directly into lactic acid from cultures on MEA and then observed under a Zeiss Axioskop bright field microscope. Mature ascomata were crushed, coated with gold, and observed and photographed with a Zeiss Supra 40 (extra high tension = 3 Kv; working distance = 3.7–6 mm) scanning electron microscope.

Molecular study

Extraction: DNA was extracted directly from colonies on potato dextrose agar (PDA) after 7–10 d at 25 °C in darkness, through the modified protocol of Müller & al. (1998). The following genetic markers were sequenced: fragments of ß-tubulin (BenA), calmodulin (CaM), RNA polymerase II second largest subunit (rpb2) (Peterson & al. 2010, Samson & al. 2014), and the DNA replication licensing factor (Mcm7) (Schmitt & al. 2009, Raja & al. 2011). The primers used were Bt2a and Bt2b for BenA, CMD5 and CMD6 for CaM (Glass & Donaldson 1995), RPB2-5F and RPB2-7R for rpb2 (Liu & al. 1999), and Mcm7-709For and Mcm7-1348Rev for Mcm7 (Schmitt & al. 2009). PCR products were sequenced in both directions using the same primers at Macrogen Europe (Macrogen Inc., Madrid, Spain). Sequences were assembled and edited using Sequencher v.4.1.4. The sequences generated were deposited in the GenBank database (https:// www.ncbi.nlm.nih.gov/genbank) using the Webin platform of the European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/ena).

Phylogenetic analysis: Multiple sequence alignment was conducted using ClustalW (Thompson & al. 1994) and MUSCLE (Edgar 2004) within MEGA v.6 software (Tamura & al. 2013), with manual adjustments for refinement. The Maximum Likelihood (ML) phylogenetic method was also run with MEGA v.6, as well as the estimation of the best nucleotide substitution model. Support of the internal branches was assessed by the bootstrap method with 1000 replications, where values ≥70 were considered significant. The phylogenetic analyses were carried out first individually for each gene followed by a concatenated study. To support our analyses, sequences were obtained from GenBank and added to the analyses.

Taxonomy

In our study, 34 heat-resistant fungal strains from the semiarid region in Argentina were all morphologically characterized and assigned to 17 taxa in 10 genera—*Arthrinium* (1), *Aspergillus* (3), *Epicoccum* (1), *Gilmaniella* (1), *Hamigera* (2), *Leiothecium* (1), *Penicillium* (2), *Talaromyces* (4), *Trichocladium* (1), and *Trichoderma* (1). For 11 strains, the morphological identification was confirmed by molecular analyses. Five taxa are reported for the first time in Argentina.

Arthrinium phaeospermum (Corda) M.B. Ellis, Mycol. Pap. 103: 8. 1965.

Colonies on MEA, 25 °C, 7 d, floccose, covering the whole culture plate, greyish, with dark specks and areas due to the anamorph presence.

Conidia lenticular, dark brown, 9–11 × 5–7 µm, smooth with a conspicuous pale equatorial stripe.

Cultures examined—**ARGENTINA. Catamarca: RP 46**, km 70, 28°05′35″S 66°12′55″W, 859 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & RC, isolation 22.X.2011 (BAFCcult 4624); 27°35′13″S 66°22′11″W, 996 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & RC, isolation 15.XI.2011 (BAFCcult 4625).

Distribution—Worldwide, including Argentina (Broggi & al. 2007, Crous & Groenewald 2013, Domsch & al. 2007).

Habitat—Cereals and vegetable materials, dung, fresh water, and soil (Domsch & al. 2007).

Comments—Our isolates agreed morphologically with descriptions by Ellis (1971), Domsch & al. (2007), and Crous & Groenewald (2013). In Argentina, this species was isolated from soil in Buenos Aires (Cabello 1986) and cereals collected in Entre Ríos (Broggi & al. 2007, Sacchi & al. 2009).

Aspergillus flavus Link, Mag. Ges. Naturf. Freunde, Berlin 3: 16. 1809.

Colonies on MEA, 25 °C, 7 d, plane, granulose, floccose at the centre, 50–54 mm diam., yellowish green, exudate scarce uncolored; asexual sporulation abundant. On Czapek yeast agar (CYA), 25 °C, 7 d, sulcate, granulose, floccose at the centre, 60-68 mm diam., yellowish green, exudate scarce uncolored; asexual sporulation abundant.

Conidial heads commonly columnar, radiate, mainly uniseriate. Stipes roughened, vesicles spherical. Conidia globose, 3.5–4.5 µm, with finely roughened walls.

Culture examined—**ARGENTINA. La Rioja: RN 60**, km 1172, 28°40′30″S 66°30′23″W, 653 m a.s.l., 12.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4615).

Distribution—Worldwide and cosmopolitan (Domsch & al. 2007; Ramírez-Camejo & al. 2012).

Habitat—Soil, cultivated or not (Domsch & al. 2007). This is the most widely cited fungal species as a food contaminant (Klich 2002, Pitt & Hocking 2009).

Comments—The micromorphology of our isolate, and even its cultural features, agreed with the descriptions of Klich (2002) and Pitt & Hocking (2009). Horn & al. (2009) described the sexual stage for this heterothallic species as *Petromyces flavus* B.W. Horn & al., but according to the International Code of Nomenclature (Turland & al. 2018) and following Samson & al. (2014), the correct name for this species is *Aspergillus flavus*. In Argentina,

it has been isolated several times from soil (Allegrucci & al. 2009, Cabello 1986, Godeas & al. 1977, Mangiaterra & al. 2006, Winitzky 1948), foods (Bresler & al. 1991, Castellari & al. 2015, Dalcero & al. 1998, Etcheverry & al. 1999, Romero & al. 2005, Sepúlveda & Piontelli 2005) and medicinal herbs (Sánchez & al. 2006).

Aspergillus montevidensis Talice & J.A. Mackinnon,
C.r. Seanc. Soc. Biol. 108: 1007. 1931. FIG. 1A,B

COLONIES on MEA, 25 °C, 7 d, plane and dense, 14 mm diam., yellowish; after 14 incubation d 26–28 mm diam., yellowish, with or without brown center. On Czapek yeast agar with 20% sucrose, 25 °C, 7 d, velutinous, 35–47 mm diam., yellow, with green center due to the anamorph presence; after 14 incubation d covering the whole culture plate, velutinous to granulose due to the abundant cleistothecia production, yellow with green center; asexual sporulation copious; reverse yellowish under cleistothecia and greenish under anamorph structures. At 37 °C, 7 d, 18–26 mm diam., yellow.

CLEISTOTHECIA superficial, mainly globose, discrete, 90–150 µm diam., yellow, covered by a hyaline hyphal net. ASCI globose, 8-spored, 10–12 × 8–11 µm, evanescent. ASCOSPORES lenticular, 5–4 × 3–4 µm, with rough convex surfaces, furrowed and double-crested along the equator; SEM images revealed pores distributed in the whole ascospore surface, but mainly on crests. CONIDIAL HEADS radiate, uniseriate. STIPES 150–210 × 6–8 µm, smooth, vesicles globose, 15–27 µm diam. PHIALIDES ampulliform, 5–8 × 4 µm. CONIDIA globose to subglobose, 5–4 × 4–3 µm, spinulose.

CULTURES EXAMINED—**ARGENTINA. CATAMARCA: RN 46**, km 185, 27°45'12"S 66°47'59"W, 1069 m a.s.l.. 9.I.2009, leg. SMR, identified by SMR, isolation 21.III.2011 (BAFCcult 4613; GenBank LT964777, LT964778); **Papachacra**, 2564 m a.s.l., 10.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4614; GenBank LT964779, LT964780).

DISTRIBUTION—Argentina (Winitzky 1952), Australia, Brazil, India, Israel, Japan, Pakistan, Peru, South Africa, Syria, Tuamotu Archipelago, Turkey (Domsch & al. 2007).

HABITAT—Soil, stored and/or decaying food products among other substrates (Domsch & al. 2007, Pitt & Hocking 1997).

COMMENTS—The morphological features of our strains agreed with those described by Thom & Raper (1941), Raper & Fennell (1965), Klich (2002) and Hubka & al. (2013). This species was also known as *Eurotium amstelodami* because Thom & Raper (1941) changed the original species

concept of Mangin (1909), from a species with smooth-walled ascospores to a species with ornamented ones. Their 1941 published name, *E. amstelodami* was, in fact, a later illegitimate homonym. Pitt (1985) noted this confusion and speculated that Thom & Church (1926) and Thom & Raper (1941) did not base their description on the Mangin species concept. Hubka & al. (2013) concluded that the first available and validly published name for the species matching the concept of Thom & Raper (1941) is *A. montevidensis*, which includes both the anamorph and the teleomorph states.

Aspergillus sydowii (Bainier & Sartory) Thom & Church, Aspergilli: 147. 1926.

Colonies on MEA, 25 °C, 7 d, velutinous to slightly floccose, 14–15 mm diam., blue green, asexual sporulation copious. On CYA, 25 °C, 7 d, sulcate, velutinous, 20 mm diam., blue green, exudate brown copious; asexual sporulation abundant; reverse orange brown. On 25% glycerol nitrate agar (G25N), 25 °C, 7 d, plane and dense, 11–13 mm diam., bluish grey.

Conidial heads radiate, biseriate; small and short penicillate heads produced. Stipes 250–350 µm, pigmented. Conidia globose, 3.5–4 µm, very rough to spinose. Hülle cells abundantly produced.

Cultures examined—**ARGENTINA. Catamarca: Rincón**, 28°22′03″S 66°13′39″W, 8.I.2009, leg. SMR, identified by SMR, isolation 4.VI.2009, (BAFCcult 4589); **RP 25**, 28°22′41″S 66°11′50″W, 1304 m a.s.l., 23.VIII.2011, leg. SMR, identified by SMR, isolation 10.II.2012 (BAFCcult 4612).

Distribution—Argentina, Australia, Austria, Brazil, British Isles, Canada, Czechoslovakia, Egypt, India, Italy, Japan, Kuwait, Malaysia, Namibia, Pakistan, Peru, Singapore, South Africa, Spain, Syria, ex USSR, U.S.A. (Domsch & al. 2007).

Habitat—Mainly isolated from soil, but also from air (indoor and outdoor) (Klich 2002). This species has been reported from numerous foods, especially dried ones (Pitt & Hocking 2009). It has been recovered also from marine sponges (Ein-Gil & al. 2009).

Comments—The features of our isolates matched those described by Raper & Fennell (1965) and Klich (2002). In Argentina, *A. sydowii* has been previously isolated from air (Winitzky 1948), amaranth seeds (Bresler & al. 1995), dried vine fruits (Romero & al. 2005), corn and soybean seeds (Sepúlveda & Piontelli 2005), soil (Allegrucci & al. 2007, Elíades & al. 2006a, Mangiaterra & al. 2006) and medicinal herbs (Sánchez & al. 2006). This is the first report for Catamarca province.

Epicoccum nigrum Link, Mag. Gesell. Naturf. Freunde Berlin 7: 32. 1816.

Colonies on Blakeslee's MEA, 25 °C, 7 d, floccose, 53–58 mm diam., orange brown, exudate and soluble pigment absent; reverse orange. On oatmeal agar (OA), 25 °C, 7 d, lanose to floccose, 60 mm diam., orange with yellowish centre, exudate absent, soluble pigment yellow; reverse yellowish orange; after 12 d of incubation brownish green centre due to sporulation. On PDA, 25 °C, 7 d, 23–30 mm diam., orange brown, exudate red caramel, soluble pigment amber; reverse red brown.

Conidiophores crowded, even forming pustules. Conidia globose to pyriform 22–30 × 18–29 µm, orange brown to dark brown, verrucose, several septa which divide the conidia in different directions, pale extending cell at the base.

Culture examined— **ARGENTINA. Catamarca: Papachacra**, 2564 m a.s.l., 10.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4622).

Distribution—Worldwide (Domsch & al. 2007).

Habitat—Cereals and grains, fruits, soil, among others (Domsch & al. 2007).

Comments—The morphologic characteristics of our isolate agreed with the descriptions of *Epicoccum nigrum* by Ellis (1971) and Domsch & al. (2007). In Argentina there are numerous records of this species, which were isolated from soil samples of Jujuy province (Giusiano & al. 2002), wheat and soybean of Entre Ríos province (Broggi & al. 2007), soil and litter of Buenos Aires province (Allegrucci & al. 2005, 2007), and alpataco fruits from La Pampa province (Canafoglia & al. 2007), among others.

Gilmaniella humicola G.L. Barron, Mycologia 56: 514. 1964. Fig. 1P,Q

Colonies on MEA, 25 °C, 7 d, velutinous to slightly floccose, 59–75 mm diam., grayish, darkening in age; reverse very dark to black. At 30 °C, 7 d, velutinous to slightly floccose, 79–83 mm diam., grayish, darkening in age; reverse very dark to black.

Hyphae light brown in age, smooth. Conidia globose, terminal or lateral, usually in clusters and provided with a distinct germ pore, smooth, brown 7–9 µm.

Cultures examined—**ARGENTINA. Catamarca: RP 25**, 28°15′31″S 66°08′47″W, 1424 m a.s.l., 23.VIII.2011, leg. SMR, identified by SMR & RC, isolation 11.I.2012 (BAFCcult 4610, 4588).

Distribution—Canada, Egypt, England, France, India, Japan, Namibia, Solomon Islands, The Netherlands, United States of America (Domsch & al. 2007).

FIG. 1. ***Aspergillus montevidensis*** (BAFCcult 4613): A. Colonies on Czapek yeast extract agar (CYA) with 20% of sucrose, 14 d, 25 °C; B. Ascospore (SEM). ***Hamigera paravellanea*** (BAFCcult 4605): C. Colonies on CYA, 7 d, 25 °C; D. Ascospores (SEM); E. Conidiophore and conidia. ***Hamigera terricola*** (BAFCcult 4580): F. Colonies on CYA, 7 d, 25 °C; G–H. Conidiophores and conidia; I. Ascospores (SEM). ***Leiothecium ellipsoideum*** (BAFCcult 4598): J. Colony on MEA, 7 d, 25 °C; K, L. Ascospores (SEM); M. Chlamydospore. ***Penicillium capsulatum*** (BAFCcult 1627): N. Colonies on CYA, 7 d, 25 °C; O. Conidiophore and conidia. ***Gilmaniella humicola*** (BAFCcult 4588): P. Conidia with germination pores (arrows); Q. Colony on MEA 7 d, 30 °C. ***Trichocladium pyriforme*** (BAFCcult 4623): R. Colony on MEA 14 d, 25 °C; S, T. Chlamydospores.

Habitat—Dung, groundnuts, plant debris, soil, among others (Domsch & al. 2007).

Comments—Our isolates were phenotypically in accordance with Barron (1964) and Ellis (1971). *Gilmaniella humicola* is a good representative example of a heat-resistant fungus (Domsch & al. 2007), and it was the most thermal-resistant fungus found by Bollen (1969), tolerating a temperature of 90 °C for 30 minutes. All our isolates were recovered from samples collected in winter. In Argentina, the fungus was previously reported from soil in a *Nothofagus dombeyi* forest in Río Negro province (Godeas 1977).

Hamigera paravellanea S.W. Peterson, Jurjević, Bills, Stchigel, Guarro & F.E. Veja, Mycologia 102: 852. 2010. Fig. 1C–E

Colonies on MEA, 25 °C, 7 d, plane somewhat floccose, 47–67 mm diam., pale orange to tan; reverse dark, mainly at colony centre. On CYA, 25 °C, 7 d, low, slightly sulcate, 50–70 mm diam., pale orange to tan; reverse dark reddish-brown.

Gymnothecia globose, discrete, 120–280 µm diam., cream coloured, surrounded by a profuse hyphal net. Asci subglobose to claviform, 8-spored, 15–20 × 11–12 µm, evanescent. Ascospores broadly ellipsoidal, 7–7.5 × 5–6 µm, hyaline, thick-walled, echinulate. Conidiophores penicillate, 120–480 × 3–5 µm. Stipes slightly spathulate, smooth or roughened towards their bases, widest at their apices. Metulae with wide apices, 10–15 × 4–6 µm. Phialides ampulliform, 8–6 × 2–3 µm. Conidia ellipsoidal, 3–4 × 2–3 µm., smooth.

Cultures examined—**ARGENTINA. Catamarca: RP 25**, 28°15′31″S 66°08′47″W, 1424 m a.s.l., 6.I.2009, leg. SMR, identified by SMR, AMS & ERA, isolation 20.IX.2011 (BAFCcult 4605, FMR 16758; GenBank LT991984, LT991994); 23.VII.2011, leg. SMR, identified by SMR, AMS & ERA, isolation 11.I.2012 (BAFCcult 4606, FMR 16762; GenBank LT992033, LT992032).

Distribution—Poland, Spain (Peterson & al. 2010).

Habitat—Soil, dung (Peterson & al. 2010).

Comments—Morphological characters observed agreed with the description of the protologue (Peterson & al. 2010). This is the first report for Argentina.

Hamigera terricola S.W. Peterson, Jurjević, Bills, Stchigel, Guarro & F.E. Veja Mycologia 102: 855. 2010. Fig. 1F–I

Colonies on MEA, 25 °C, 7 d, low, sulcate, velutinous, 58–70 mm diam., ochraceous buff to tan; reverse dark reddish-brown. On CYA, 25 °C, 7 d, low,

plane, velutinous, 40–43 mm diam., ochraceous buff with yellowish margins; reverse dark reddish-brown.

Gymnothecia globose, discrete, 80–180 µm diam., cream coloured, surrounded by a profuse hyphal net. Asci subglobose, 8-spored, 15–17.5 × 11–13 µm, evanescent. Ascospores broadly ellipsoidal, 7–8 × 5–6 µm, hyaline, thick-walled, echinulate. Conidiophores penicillate, 170–400 × 3–6 µm. Stipes sometimes spathulate, with apical vesicles 7–9 µm wide, smooth or slightly roughened. Metulae with wide apices, 7–11 × 3–6 µm. Phialides ampulliform, 7–8 × 3 µm. Conidia ellipsoidal, 3–4 × 2–3 µm., smooth.

Culture examined—**ARGENTINA. Catamarca: RN 60,** km 1102, 28°55′15″S 66°08′46″W, 338 m a.s.l., 5.I.2009, leg. SMR, identified by SMR, AMS & ERA, isolation 4.VI.2011 (BAFCcult 4580, FMR 16756; GenBank LT991985, LT991995).

Distribution—Costa Rica, Equatorial Guinea, French Guiana, Guatemala, Panama (Peterson & al. 2010).

Habitat—Textile sample in contact with soil (Peterson & al. 2010).

Comments—In a broad sense, the features of this isolate matched with those described by Peterson & al. (2010), but ascospores are slightly larger and the asci smaller than in the original description. This is the first report of *Hamigera terricola* for Argentina.

Leiothecium ellipsoideum Samson & Mouch.,
Can. J. Bot. 53: 1634. 1975. Fig. 1J–M

Colonies on MEA, 25 °C, 7 d, forming concentric rings, 82–83 mm diam., dark, superficial white mycelium present; reverse dark. At 35 °C, covering the whole culture plate before 7 d; reverse dark due to ascomata abundance. On OA, 25 °C, 7 d, colonies presenting a continuous layer of ascomata, dark, almost lacking aerial mycelium. At 35 °C, covering the whole culture plate before 7 d; subtle annularly developed, less aerial mycelium than on MEA.

Vegetative hyphae smooth, 3–11 µm diam., hyaline. Cleistothecia globose, discrete, 40–90 µm diam., dark brown, superficial or semi-submerged in the substratum, covered by white mycelium supporting ascomata as well; wall persistent, textura angularis, 5–8 µm wide, composed by 15–25 µm diam. dark brown cells. Asci globose to subglobose, 14–18 × 13–15 µm, evanescent. Ascospores ellipsoidal, 7–9 × 5.5–7 µm, hyaline, spinulose under low magnifications, broadly reticulate under high magnifications; young ascospores presented an internal hyaline sheath which fades with maturity; SEM observations revealed alveolate plaques on ascospore wall, alveoles presented conspicuous projections at their edges which look as low frills.

Chlamydospores isolated, subglobose to ellipsoidal, umbonate, 4.5–11 µm diam., smooth, endogenous, and also terminal in maturity.

Cultures examined— **ARGENTINA. Catamarca: RN 60**, km 1146, 28°13'05"S 66°22'41"W, 1051 m a.s.l., 5.I.2009, leg. SMR, identified by SMR, RC & AMS, isolation 13.IX.2011 (BAFCcult 4598, FMR 15064; GenBank LT992254, LT992257); km 1016, 29°30′04″S 65°37′57″W, 237 m a.s.l., 22.VIII.2011, leg. SMR, identified by SMR, RC & AMS, isolation 12.XI.2011 (BAFCcult 4604, FMR 16765; GenBank LT992253, LT992256); **RP 47**, km 35.5, 27°26′50″S 66°24′26″W, 2700 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR, RC & AMS, isolation 18.I.2012 (BAFCcult 4609, FMR 16768; GenBank LT992255, LT992258).

Distribution—Canada (Samson & Mouchacca 1975), Greece, Hungary, Portugal, Spain, United States of America, and Venezuela (CBS-KNAW Culture Collection 2018).

Habitat—Soil, seeds of *Capsicum annuum* (Samson & Mouchacca 1975), nest material of *Nomia* sp., a ground-nesting solitary bee (CBS-KNAW Culture Collection 2018).

Comments—Argentinian isolates largely agreed with the description given by Samson & Mouchacca (1975) and Guarro & al. (2012). However, these strains grew faster on MEA at 35 °C than the ex-type strain. Regarding micromorphology, no chlamydospores in chains were observed (solitary chlamydospores were seen on small hyphal protrusions) and ascomata were smaller (40–90 µm vs. 125 µm diam) than those described in the protologue. This is the first report of *Leiothecium ellipsoideum* for Argentina.

Penicillium capsulatum Raper & Fennell, Mycologia 40: 528. 1948. Fig. 1n,o

Colonies on MEA, 25 °C, 7 d, low, plane, velutinous, 14–18 mm diam., white mycelium at the edges, grey green to dull green; conidiogenesis moderate; exudate and soluble pigment absent; reverse pale to yellowish. On CYA, 25 °C, 7 d, moderately deep, sulcate, slightly fasciculate, 12–15 mm diam.; conidiogenesis dense, grey green to dull green, white mycelium at the edges; exudate and soluble pigment absent; reverse pale to yellowish. At 37 °C, 7 d, funiculose, 18–21 mm diam., with white mycelium at the edges; exudate and soluble pigment absent; reverse pale to buff. At 5 °C germination absent. On G25N, 25 °C, 7 d, sulcate, velutinous to floccose, 6 mm diam., white mycelium at the edges, conidia in mass grey green to dull green; exudate and soluble pigment absent; reverse pale to yellowish.

Teleomorph absent. Conidiophore monoverticillate, arising from substrate surface or from superficial mycelium. Stipes somewhat sinuose, 10–40 µm long., smooth, mainly non-vesiculate, but sometimes vesicles up to

4 µm diam. Phialides 5–8, ampulliform to acerose, 6 × 2 µm, occasionally up to 8 µm in length, with short collula. Conidia ellipsoidal to cylindrical, 3–3.5 × 2 µm, smooth-walled.

Culture examined—**ARGENTINA. Catamarca: RP 33**, 28°42′03″S 65°46′08″W 418 m a.s.l., 22.VIII.2011, leg. SMR, identified by SMR & RC, isolation 23.XI.2011 (BAFCcult 1627).

Distribution—China (Chen & al. 2013), Kiribati (Gilbert Islands), Panamá (Raper & Fennell 1948).

Habitat—Optical instrument, environmentally exposed canvas and deteriorated military equipment (Raper & Fennell 1948), and contaminated beet pulp (Puls & Coughlan 1996).

Comments—Our soil-borne isolate matched the Pitt (1979) description. We also observed vesiculate conidiophores, but phialides were a bit shorter than in the holotype. Pitt (1979) described the colonies on CYA at 25 °C as depressed at the center, whereas the Argentinian strains presented a high and fasciculate center. This is the first report of *Penicillium capsulatum* for Argentina.

Penicillium citrinum Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 61. 1910.

Colonies on MEA, 25 °C, 7 d, low, plane, centrally umbonate, velutinous, 18–22 mm diam., grey green to dull green; conidiogenesis dense; exudate pale, soluble pigment absent; reverse pale. On CYA, 25 °C, 7 d, moderately deep, sulcate, velutinous, 24–30 mm diam., grey green to dull green; conidiogenesis dense, white mycelium at the edges; exudate pale, soluble pigment yellowish; reverse yellow to orange. At 37 °C, 7 d, 2–11 mm diam. At 5 °C no germination.

Teleomorph absent. Conidiophores biverticillate, arising from substrate surface or from superficial mycelium. Stipes with smooth walls and a conspicuous divaricate metulae whorl. Metulae of uniform length, 3–5 on each stipe. Phialides ampulliform, short collula. Conidia globose to subglobose, 2.5–3 µm diam., smooth-walled.

Culture examined—**ARGENTINA. Catamarca: Corral Quemado to Papachacra**, 27°07′33″S 66°56′36″W, 2152 m a.s.l.: 10.I.2009, leg. SMR, identified by SMR & RC, isolation 5.III.2011 (BAFCcult 4616).

Distribution—*Penicillium citrinum* is considered a cosmopolitan and frequently isolated fungus (Domsch & al. 2007).

Habitat—The fungus has been reported from many substrates, including food (Domsch & al. 2007).

Comments—Our isolate matched with the description of *Penicillium citrinum* provided by Pitt (1979). In Argentina, it has been isolated many times, generally

from food, e.g., soybeans (Bonera & al. 1982), amaranth seeds (Bresler & al. 1991, 1995), balanced feed for birds (Magnoli & al. 1998), and dried vine fruits (Romero & al. 2005), among others.

Talaromyces macrosporus (Stolk & Samson) Frisvad, Samson & Stolk, Antonie van Leeuwenhoek 57: 186, 1990.

Colonies on MEA, 25 °C, 14 d, almost covering the whole culture plate, moderate deep, plane, granulose, covering the whole culture plate, yellow, becoming orange at the centre.

Cleistothecia superficial, mainly globose, commonly confluent but at the margin discrete, 200–400 µm diam., yellow. Asci globose, 8-spored, evanescent; initials showing thin antheridia coiled around slender clavated ascogonia. Ascospores ovoidal to broadly ellipsoidal, 5.5–6.5 × 3.5–4 µm, pale yellow, with thick walls and spines. Anamorph absent.

Culture examined—**ARGENTINA. Catamarca: RP 33**, 28°42′03″S 65°46′08″W, 418 m a.s.l., 22.VII.2011, leg. SMR, identified by SMR & AIR, isolation 23.XI.2011 (BAFCcult 4617).

Distribution—Ghana, Japan, Korea, Malaysia, New Guinea, Panama, Poland, South Africa, The Netherlands, United States of America (CBS-KNAW Culture Collection 2018, Stolk & Samson 1972).

Habitat—Soil, canned apples (Stolk & Samson 1972), freshly harvested strawberries (Frisón & al. 2012).

Comments—Our isolate agreed with the description of *Talaromyces macrosporus* presented by Stolk & Samson (1972), who recognize two varieties: *T. flavus* var. *flavus* and *T. flavus* var. *macrosporus*. Regarding these varieties, Beuchat (1988) pointed out that the strains with small ascospores (*T. flavus* var. *flavus*) are less thermal resistant than those with larger ones (*T. flavus* var. *macrosporus*). Frisvad & al. (1990) raised them to species rank based on ascospore size, heat-resistance, and secondary metabolite production. In Argentina, the species has previously been recorded in soils either as *T. flavus* s.l. or as *T. flavus* var. *flavus* (Bertoni 1973, Elíades & al. 2006 a,b, Magnoli & al. 1998). Frisón & al. (2012) reported *T. macrosporus* on fresh strawberries.

Talaromyces pinophilus (Hedgc.) Samson, N. Yilmaz, Frisvad & Seifert, Stud. Mycol. 70: 176. 2011.

Colonies on MEA, 25 °C, 7 d, moderately deep, plane, floccose, 34–36 mm diam., green; conidiogenesis sparse, yellow mycelium present, almost dominating colony colour; exudate pale, soluble pigment absent; reverse

yellowish. On CYA, 25 °C, 7 d, moderately deep, plane, floccose, 19–20 mm diam., greenish yellow; conidiogenesis sparse, yellow mycelium present predominating in colony colour; exudate pale, soluble pigment absent; reverse brown. At 37 °C, 7 d, 24–26 mm diam., with white mycelium at the edges; exudate and soluble pigment absent; reverse buff. At 5 °C no germination. On G25N, 25 °C, 7 d, plane, floccose, 3–5 mm diam., white mycelium; sporulation absent; exudate pale, soluble pigment absent; reverse buff.

Teleomorph absent. Conidiophores biverticillate, arising from substrate surface and also from aerial hyphae. Stipes 100–150 µm long., smooth. Metulae in whorls mainly of 5 elements, 10–12 µm long. Phialides 5–8, acerose, 8–10 µm long., tapered. Conidia globose to subglobose, 3–4 µm diam., smooth-walled.

Culture examined—**ARGENTINA. Catamarca: Corral Quemado to Papachacra**, 27°07′33″S 66°56′36″W, 2152 m a.s.l., 10.I.2009, leg. SMR, identified by RC, isolation 5.III.2011 (BAFCcult 4618).

Distribution—Argentina (Magnoli & al. 1998); Australia, France, India, Papua New Guinea, United States of America (Pitt 1979); Uruguay (Galvalisi & al. 2012).

Habitat—Soil, decaying plants, deteriorated material, barley grains, fermented dry sausages, balanced feed for birds (references as above).

Comments—Excluding conidia, which in this case were a little bit bigger (in contrast to 2.5–2.8 × 2.2–2.5 µm), we did not find morphological differences compared to those of *Talaromyces pinophilus* given by Pitt (1979). In Argentina, it has been previously reported as *Penicillium pinophilum* from corn, balanced feed for birds, and soil (Etcheverry & al. 1999, Magnoli & al. 1998, Nesci & al. 2006, Mangiaterra & al. 2006).

Talaromyces trachyspermus var. ***macrocarpus*** J.E. Wright & Loewenb., Bol. Soc. Argent. Bot. 15(1): 100. 1973.

Colonies on MEA, 25 °C, 14 d, moderately deep, plane, granular, 37–44 mm diam., white to cream-colored; exudate absent; reverse orange.

Cleistothecia superficial, mainly globose, usually confluent, 400–930 µm diam., at first white becoming cream-colored in age, covered by a network of hyphal net. Asci globose to ovoid, 8-spored, 7–8 × 6–7 µm, evanescent. Ascospores ellipsoidal, 3–4 × 2–3 µm, spinulose. Conidiophores slender monoverticillate and biverticillate, short. Metulae 8–11 × 2 µm. Phialides 2–5 in verticils, lanceolate, tapering to neck, 9–10 × 2 µm. Conidia ellipsoidal, 2–2.5 × 2 µm, smooth-walled.

Culture examined—**ARGENTINA. Catamarca: RP 46**, km 185, 27°45′12″S 66°47′59″W, 1069 m a.s.l., 9.I.2009, leg. SMR, identified by SMR & AIR, isolation 21.III.2011 (BAFCcult 4621; GenBank LT968851).

Distribution—Argentina (Bertoni & al. 1973).

Habitat—Soil (Bertoni & al. 1973).

Comments—The characteristics of isolate BAFCcult 4621 agreed with the description of *Talaromyces trachyspermus* var. *macrocarpus* by Bertoni & al. (1973). This variety was not accepted by Pitt (1979); however, we prefer to recognize it until examination of the holotype, and study of more isolates and their molecular data. *T. trachyspermus* var. *macrocarpus* is distinguished from *T. trachyspermus* by presenting bigger cleistothecia (500–1500 vs 50–350 µm) and ascospores (4–4.5 × 2.8–3.2 vs 3–3.5 × 2–2.5 µm).

Talaromyces trachyspermus (Shear) Stolk & Samson, Stud. Mycol. 2: 32 (1973) var. ***trachyspermus***

Colonies on MEA, 25 °C, 14 d, moderately deep, plane, granular, 42–50 mm diam., white to cream-colored; exudate absent; reverse light orange and orange to brown in the centre.

Cleistothecia superficial, mainly globose, usually confluent, 150–350 µm diam., at first white becoming cream-colored in age, covered by a hyphal network. Asci globose to ovoid, 8-spored, 7–9 × 6–8 µm, evanescent. Ascospores ellipsoidal, 3.5–4 × 2–3 µm, spinulose. Conidiophores slender monoverticillate and biverticillate, short. Metulae 8–11 × 2 µm. Phialides 2–5 in verticils, lanceolate, tapering to neck, 9–10 × 2 µm. Conidia ellipsoidal, 2–2.5 × 2 µm, smooth-walled.

Cultures examined—**ARGENTINA. Catamarca: RP 46**, 27°35′13″S 66°22′11″W, 996 m a.s.l., 9.I.2009, leg. SMR, identified by SMR & AIR, isolation 21.III.2011 (BAFCcult 4619; GenBank LT968849); (BAFCcult 4620; GenBank LT968850); 28°46′22″S 66°09′56″W, 1317 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & AIR, isolation 4.I.2012 (BAFCcult 4629); **Belén**, 27°07′33″S 66°56′36″W, 2152 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR, isolation 27.I.2012 (BAFCcult 4630); **RP 47**, km 35.5, 27°26′50″S 66°24′26″W, 2700 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR, isolation 18.I.2012 (BAFCcult 4631).

Distribution—Argentina, Bangladesh, France, Germany, India, Japan, Nepal, Pakistan, South Africa, Tahiti, Uganda, United States of America (Domsch & al. 2007).

Habitat—Soil, cereals and grains, dung (Domsch & al. 2007).

Comments—The morphological features of our isolates agreed with the original description of *Talaromyces trachyspermus* (Stolk & Samson 1972). In

Argentina Bertoni & al. (1973) and Godeas (1975) isolated this fungus from soil.

Trichocladium pyriforme M. Dixon,
Trans. Br. Mycol. Soc. 51: 160. 1968. FIG. 1R–T

COLONIES on MEA, a 25 °C, 7 d, plane, slightly floccose, 36–40 mm diam., grey green to olive, darkening in age; reverse very dark; covering the whole culture plate in 21 d.

HYPHAE hyaline when young, yellow brown in age, 2–5 µm wide, smooth. CHLAMYDOSPORES (holothallic conidia) terminal, pyriform, 3-4 celled, 16–19 × 6–7.5 µm, smooth-walled; distal cell dark brown, with an acute apex and germinative pore.

CULTURES EXAMINED—**ARGENTINA. CATAMARCA: RN 60**, km 934, 29°33′35″S 64°52′56″W, 186 m a.s.l., 5.I.2009, leg. SMR, identified by SMR & AIR, isolation 5.I.2011 (BAFCcult 4623); **RN 40**, km 4243.5, 26°51′23″S 66°05′52″W, 2031 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR & AIR, isolation 12.XI.2011 (BAFCcult 4611).

DISTRIBUTION—Czech Republic (Mantle & al. 2006), Ireland (Dixon 1968).

HABITAT—Soil (Dixon 1968, Goh & Hyde 1999).

COMMENTS—The morphological features of the Argentinian strains of *Trichocladium pyriforme* agreed with those described by Dixon (1968) and by Goh & Hyde (1999). Domsch & al. (2007) pointed out the remarkable thermal resistance of the spores of this species, and Dixon (1968) stated that it tolerated up to 95 °C for 10 min. Bollen (1969) isolated *T. pyriforme* from greenhouse soil using a thermal shock at temperatures greater than 55 °C. This is the first report of this fungus in Argentina.

Trichoderma saturnisporum Hammill, Mycologia 62: 112. 1970.

COLONIES on special nutrient agar (SNA), 72 h, 30 °C, in darkness, somewhat annellated, 38–44 mm diam., green. At 35 °C, 72 h, 38 mm diam. On PDA, 72 h, 30 °C, slightly annellated, 60 mm, dull green; at 35 °C, 69 mm diam. in darkness.

CONIDIOPHORES associated in tufts up to 4 mm diam., branched, short sterile projections present. PHIALIDES mainly in verticils, cylindrical, 8.5–11 × 3.3–4 µm. CONIDIA subglobose to broadly ellipsoidal, 4–4.8 × 2.9–3.7 µm, greenish, walls with few notorious warts. CHLAMYDOSPORES not observed.

CULTURES EXAMINED—**ARGENTINA. CATAMARCA: RN 60,** km 1016, 29°30′04″S 65°37′57″W, 237 m a.s.l., 5.I.2009, leg. SMR, identified by SMR & RC, isolation 3.VIII.2010 (BAFCcult 4584, 4586, 4587); **RP 25**, 28°14′36″S 66°08′51″W, 1507 m a.s.l., 6.I.2009, leg. SMR, identified by SMR & VB, isolation 1.XI.2010 (BAFCcult 4626);

Rincón, 28°22′03″S 66°13′39″W, 8.I.2009, leg. SMR, identified by SMR & VB, isolation 19.VIII.2010 (BAFCcult 4627, 4628.

Distribution—Argentina (Godeas & al. 1977), Australia, Italy, South Africa, Turkey, United States (Samuels & al. 1998).

Habitat—Soil.

Comments—The morphological features of the examined culture agreed with those given by Samuels & al. (1998). *Trichoderma saturnisporum* is easily recognized by conidia with large warts mimicking wings, which give a saturnian appearance to the conidia.

Discussion

In previous papers we described two new species of *Eurotiales*, *Talaromyces systylus* S.M. Romero & al. (Romero & al. 2016) and *Aspergillus fuscicans* S.M. Romero & al. (Romero & al. 2018) from the same locations and substrate (soil) as in the present work. The isolates in *Aspergillus* sect. *Fumigati*, which were prevalent in the analyzed samples, are currently under study.

Some of the fungal species found have been previously cited as heat-resistant, such as *Arthrinium phaeospermum* (Kashiwagi & al. 2009, Pitt & Hocking 2009), *Gilmaniella humicola* (Bollen 1969, Jesenská & al. 1992), *Leiothecium ellipsoideum* (Samson & Mouchacca 1975), *Talaromyces macrosporus* (Beuchat 1988, Frisón & al. 2012) and *Trichocladium pyriforme* (Bollen 1969, Dixon 1968).

It was not possible to obtain information about the heat-resistance of *Aspergillus montevidensis*; however, *Aspergillus glaucus* (L.) Link, another species in *A.* sect. *Aspergillus*, was isolated from contaminated jellies and grape marmalades, and its thermal resistance was analyzed (Splittstoesser & al. 1989). No information about the heat resistance was found for *Trichoderma saturnisporum*; however, chlamydospore production (Samuels & al. 1998) would explain its survival after a thermal shock performed in the lab.

Byssochlamys nivea, perhaps the most well-known heat-resistant fungal species, was not isolated during this study. This could be attributed to our thermal treatment (75 °C, 30 min), since other authors have determined that its ascospores can survive at 70 °C for 60–75 min but not at 80 °C for 1 min (Piecková & al. 1994). The same authors also mentioned that at 80 °C the ascospores of *Hamigera avellanea* Stolk & Samson survive 120 min, whereas the conidia of *Gilmaniella humicola* survive between 8–14 min. Although no data were found on the thermal resistance of *H. paravellanea* and *H. terricola* ascospores, given the heat resistance described for *H. avellanea* (Piecková & al.

1994, Scaramuzza & Berni 2014), it can be assumed that other species of the genera would possess a similar capacity.

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Cortinarius rapaceoides, a new record for Turkey

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Abstract—A macrofungus collected from Akbelen village in Tokat has been identified based on molecular evidence and morphology as *Cortinarius rapaceoides,* a new record from Turkey. Sequence data generated from the nuclear ribosomal internal transcribed spacer and ribosomal large subunit regions were deposited in GenBank and used to determine phylogenetic relationships within *Cortinarius* subg. *Phlegmacium*. A morphological description, illustrations, and phylogeny of the Turkish *C. rapaceoides* are provided.

Key words—*Cortinariaceae*, ITS, LSU, taxonomy

Introduction

The ectomycorrhizal basidiomycete genus *Cortinarius* (*Agaricales*) is the world's most speciose mushroom genus. Ectomycorrhizal associations formed with trees (e.g., *Nothofagus, Quercus, Pinus*) and herbaceous plants are important for forest ecosystem (Moser & Horak 1975, Breitenbach & Kränzlin 2000, Stensrud & al. 2014, Itoo & al. 2015). Morphological features such as cortina covering young gills, rusty brown mature gills, ochraceous-rusty to deep rusty spore print, and bulbous stipe distinguish this genus. Species separation within *Cortinarius* is greatly complicated by a high degree of inter- and intra-specific morphological variation.

Molecular markers have become important in helping resolve and refine *Cortinarius* taxonomic classification. Previous studies have used molecular markers derived from ribosomal gene loci containing the internal

transcribed spacer regions (ITS1 and ITS2), ribosomal large subunit (LSU), and the two largest subunits of RNA polymerase II (RPB1, RPB2) (Garnica & al. 2003, 2009; Froslev & al. 2005, 2007; Niskanen & al. 2009; Liimatainen & Niskanen 2013; Stensrud & al. 2014; Liimatainen & al. 2017; Sesli & Liimatainen 2018). Among those, ITS data strongly supports phylogenetic delimitation of species among *Cortinarius* sections and our understanding of the evolution of the genus (see Garnica & al. 2009). Most studies stress the necessity of combining sequence data with morphological studies to resolve the relationships among *Cortinarius* species.

Cortinarius species have been previously recorded in Turkey (Sesli & Denchev 2014; Akata & al. 2015; Güngör & al. 2015; Sesli & al. 2015, 2016; Sesli 2018; Sesli & Liimatainen 2018; Kalmer & al. 2019). Our research provides the first identification of *C. rapaceoides* in Turkey with detailed description of morphological characteristics and evidence at the molecular level.

Materials & methods

Morphological studies

Fresh *Cortinarius* basidiomes were collected from Akbelen village (Tokat) in the autumn of 2018, photographed in the field, and important morphological and ecological features noted. Basidiomes were stored in paper bags and kept in a box. The fresh collection was transported to the laboratory in paper bags, and collection numbers were assigned. The dried basidiomes were placed into polyethylene bags for storage. Tissues were mounted in Melzer's reagent, distilled water, Congo red, and KOH prior to examination under a light microscope. At least 25 measurements from each microscopic structure were obtained from a mature basidiome, which was initially identified based on its morphological features with the help of Boisselet (2012a) and Tanchaud (2016). The identified collection was deposited in the Fungarium of the Department of Biology, Tokat Gaziosmanpasa University, Tokat, Turkey (GOPUF).

Molecular studies

Genomic DNA was extracted from lamella using the ZR Fungal/Bacterial DNA MiniPrep kit as described by the manufacturer's protocol. The primer pair ITS4-ITS5 (White & al. 1990) was used to amplify ITS1-5.8S-ITS2 region and the primer pair LROR-LR5 (Vilgalys & Hester 1990) was used to amplify 28S LSU rRNA gene region. The DNA was amplified in a 30 µl volume mixture containing 3 µl 10X buffer, 3 µl dNTP mix, 3 µl degenerate primer pair (final concentration of 1 µM each), 0.3 µl Dream Taq DNA polymerase (Thermo), 10 µl gDNA and 7.7 µl sterile ddH_2O. PCR amplification for ITS region was set as: A negative control reaction using only sterile ddH_2O was also run in parallel. 5 min initial denaturation at 95°C + 40 denaturation

cycles (95°C for 30 sec) + annealing (53°C for 30 sec) + extension (72°C for 1 min) and a final extension for 10 min. The LSU PCR protocol comprised: a 3 min initial denaturation at 95°C + 40 denaturation cycles (95°C for 30 sec) + annealing (48°C for 30 sec) + extension (72°C for 1 min) and a final extension for 10 min.

PCR products were run in a 1% agarose gel electrophoresis and positive PCR products were gel purified by using Wizard SV Gel and PCR Clean-up System. Purified PCR products were sequenced in both directions using the same primer pairs by BM Labosis Inc. (Ankara).

Sequence and phylogenetic analysis

Chromatograms were checked for any nucleotide errors for each genomic sequence generated from both ends and errors were corrected manually. Assembled rDNA sequences were examined using Basic Local Alignment Search Tool (BLAST) programme to select the most closely related ITS and LSU sequences in the database. Representative ITS and LSU sequences of *Cortinarius* species were retrieved from GenBank for phylogenetic analysis. The sequence alignments and phylogenetic trees for each genomic region were analysed using MEGA 6.0 (Tamura & al. 2013). In the CLUSTALW alignment, sequences were trimmed at both ends. Phylogenetic trees were constructed using the maximum likelihood (ML) and maximum parsimony (MP) methods. Tamura-Nei model (Tamura & Nei, 1993) was used to construct the ML tree with bootstrap support of 1000 replicates and default settings. The bootstrap support values ≥50% were marked on the branches of the tree.

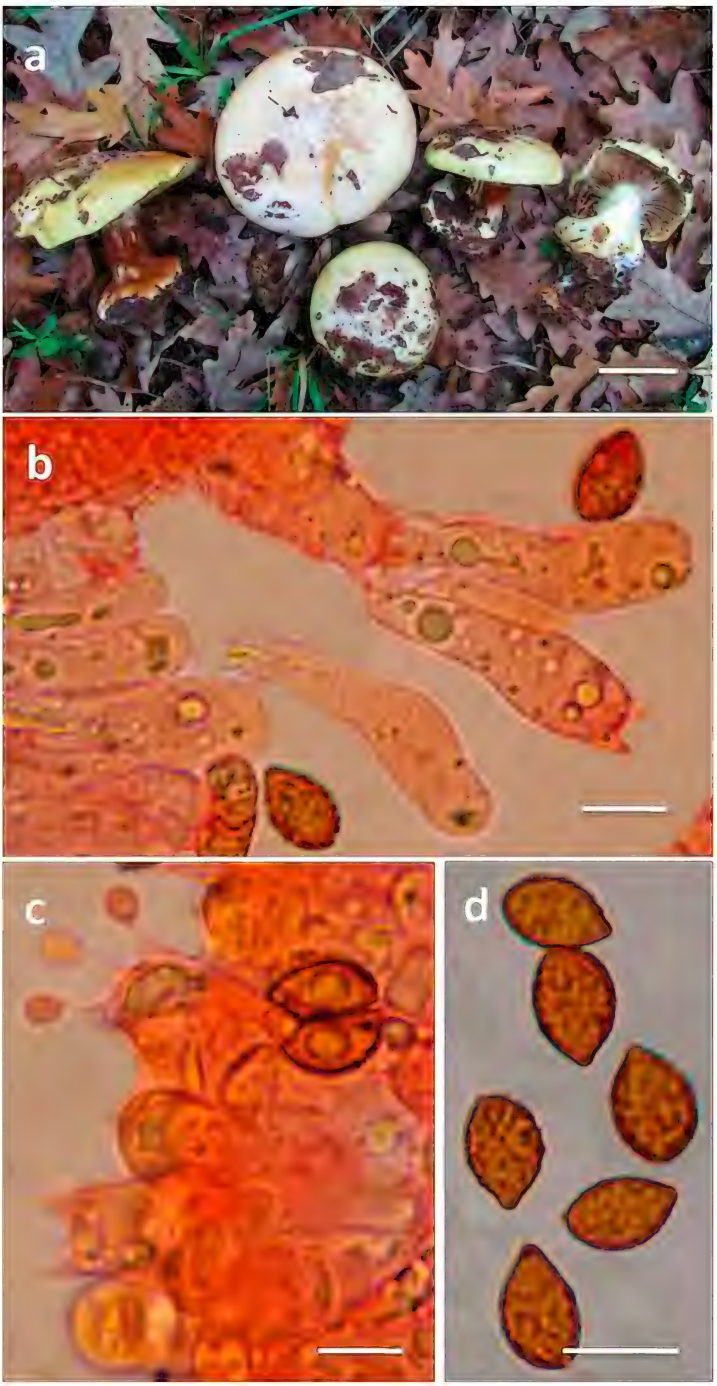
Taxonomy

Cortinarius rapaceoides Bidaud, G. Riousset & Riousset, Micologia 2000: 68 (2000) Fig. 1

Pileus 50–100(–120) mm diam., at first convex then plane-convex to plane; surface dull when dry, lubricous when moist; colour overall yellowish to ochre-yellow, in the centre orange-yellow with darker brown squamules. Lamellae pale lilac, soon rust-brown, broad, crowded, edges smooth to slightly crenate. Stipe whitish, sometimes gray-purplish at the apex, fragile, solid, cylindrical, base with a marginate bulb, surface longitudinally whitish-fibrillose when young, brownish when old. Flesh whitish sometimes bluish in the upper and lower stipe. KOH negative on the flesh, amber yellow colour on the pileal surface. Odor of chocolate.

Basidiospores (9.6–)10–13(–13.8) × (6.0–)6.5–8(–8.5) µm, elliptical to amygdaliform, strongly verrucose, yellow-brown. Basidia, (30–)35–40(–44.6) × (9–)10.5–12(–12.4) µm, with 2–4 sterigmata, clavate.

Specimens examined: **TURKEY, Tokat, Akbelen village,** 40°27′0″N 36°39′16″E, 1050 m, among dead leaves of *Quercus* sp, 8 November 2018, leg. Isik 798 (GOPUF; GenBank MN094878, MN099705).


a
b
c
d

Habitat & distribution—Among fallen leaves on calcareous soils in deciduous woods. Previously recorded from France and Italy (Liimatainen & al. 2014).

Molecular phylogeny

The ITS1-5.8S-ITS2 (666 bp) and LSU rRNA (996 bp) sequences were deposited in GenBank. ITS sequence MN094878 BLAST results matched closely with the sequences representing *C. caroviolaceus* (EU057049), *C. rapaceoides* (KF732407, ex-holotype), and *C. saporatus* (DQ663413), which are included in the /Caroviolacei clade (Garnica & al. 2009). The remaining BLAST hits were also matched with species within the same clade. Accordingly, 67 sequences in the /Calochroi & Fulvi clade of *Cortinarius* subg. *Phlegmacium* (Liimatainen & al. 2014) were selected and ITS sequences were retrieved from GenBank for phylogenetic analyses. The outgroup *Cortinarius aureifolius* and *C. caerulescens* were used to root the phylogeny (Froslev & al. 2005, Garnica & al. 2009).

Although fewer in number, LSU sequences for genus *Cortinarius* were also selected to construct a phylogenetic tree, but the LSU-based phylogeny was not well resolved due to low bootstrap values and no phylogenetic separation at the subgenus level was observed for LSU regions.

Our two constructed MP and ML phylogenetic trees showed similar topologies for each genomic region. We present only the ML tree to indicate phylogenetic relationship of the studied species based on the ITS region. The sequence from our *Cortinarius* specimen clustered with sequences derived from *C. rapaceoides* described from Italy and France (including the ex-holotype sequence KF732407) and *C. caroviolaceus* (EU057049) from Italy with high bootstrap support (Fig. 2). Liimatainen & al. (2014) suggested that *C. caroviolaceus* sequence actually represented *C. rapaceoides* based on their ITS sequence phylogeny; and our phylogeny also supports it as conspecific with *C. rapaceoides*.

Discussion

Cortinarius rapaceoides, originally described from France and collected under *Quercus ilex* in a deciduous forest with calcareous soil, is characterized by a cream to yellow color, smooth convex pileus with a rolled margin,

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Fig. 1. *Cortinarius rapaceoides* (GOPUF – Isik 798): A. basidiomata; B. basidia and basidioles; C. basidia; D. basidiospores. Scale bars: A = 3 cm; B–D = 10 µm.

cream to purple lamellae, blue, cream or purple colored cylindrical stipe with a marginate bulb, 10–13 × 6–7 µm verrucose spores, and a hardwood mycorrhizal association (Boisselet 2012a, Tanchaud 2016).

The species is morphologically similar to *C. aleuriosmus* Maire [= *C. caro-violaceus* P.D. Orton] and *C. saporatus* Britzelm. These fungi may be confused with each other due to their similar ecological features; however, *C. aleuriosmus* can be distinguished from *C. rapaceoides* by its whitish pileus and lamellae, smaller, almond-to lemon-shaped rough spores, abundant white veil, and farinaceous taste and odor (Orton 1955, Moser 1983, Phillips 1981, 2013; Boisselet 2012a, 2012b; Tanchaud 2016). *Cortinarius saporatus* differs in its bigger pileus and stipe, dark brown colour changes with KOH on the pileus, its yellowish colour at the stipe base, and an odor that is initially fruity but later more sour (Phillips 1981, 2013). Although some spores of Turkish *C. rapaceoides* are larger, the morphological characters are very similar and agree with French *C. rapaceoides* (Boisselet 2012a, Tanchaud 2016).

Sequence analyses also support identification of Turkish collection as *C. rapaceoides*. It belongs in *Cortinarius* subg. *Phlegmacium*, a polyphyletic subgenus (Peintner & al. 2004, Garnica & al. 2005). Although *Cortinarius* species are morphologically quite variable, their ITS sequences show a high sequence identity and are reliably used for species delimitation and identification (e.g. Froslev & al. 2005, 2007; Ortega & al. 2008; Garnica & al. 2009; Niskanen & al. 2009). We found the ITS region more useful than the LSU region for inferring the phylogenetic relationships and were able to determine independent phylogenetic lineages with high boostrap support. Our ITS sequence clustered in *Cortinarius* sect. *Calochroi* and showed 100% sequence identity with *C. rapaceoides* from France and Italy without any intraspecific sequence polymorphisms.

Our first record of *C. rapaceoides* in Turkey is well supported by both morphological and molecular evidence.

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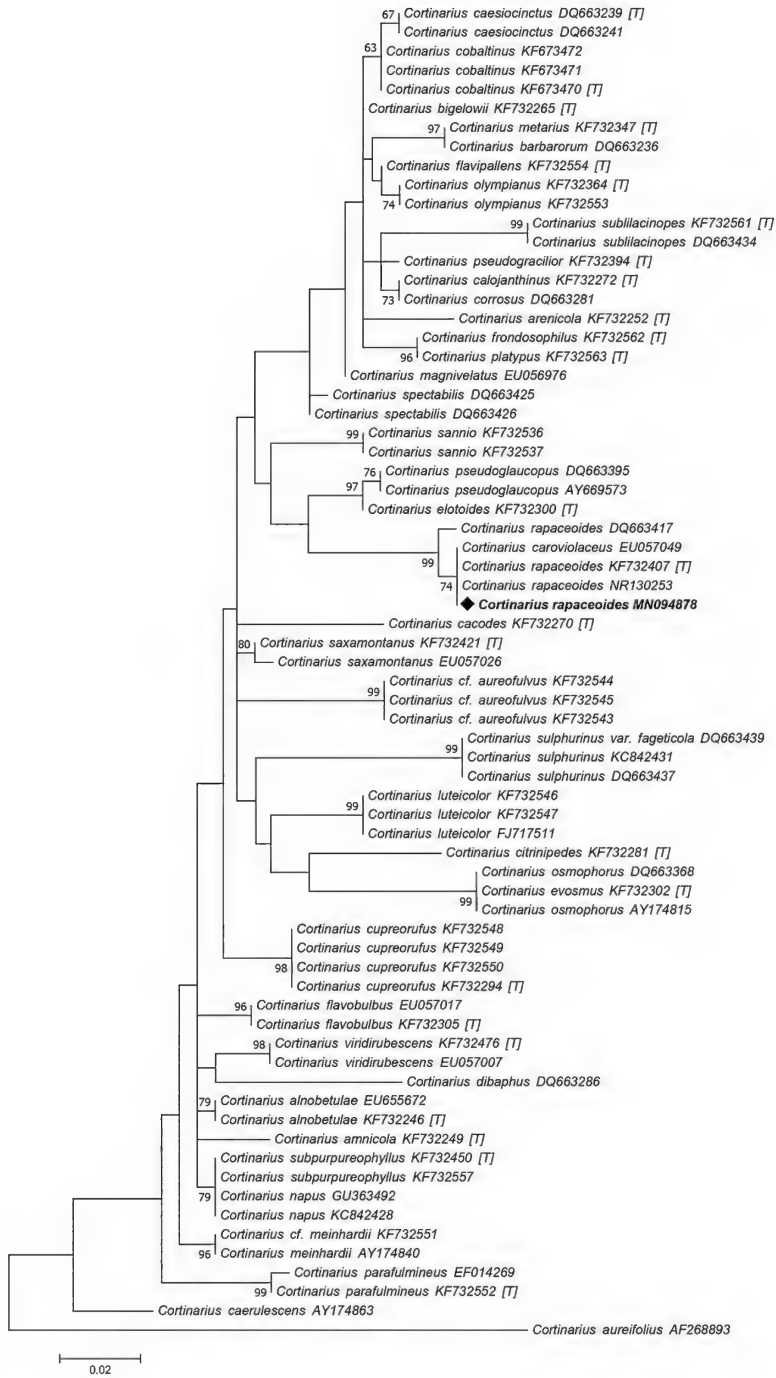
Fig. 2. Phylogeny of selected *Cortinarius* species inferred from ITS sequences using the ML method. The diamond [♦] indicates the Turkish *C. rapaceoides* identified in this study. Ex-type sequences are annotated with [T]. *Cortinarius aureifolius* and *C. caerulescens* represent the outgroup. Bootstrap support values ≥50% from ML analysis are shown on the branches. Bar = 0.02 expected changes per site per branch.


67 Cortinarius caesiocinctus DQ663239 [T]
Cortinarius caesiocinctus DQ663241
63 Cortinarius cobaltinus KF673472
Cortinarius cobaltinus KF673471
Cortinarius cobaltinus KF673470 [T]
Cortinarius bigelowii KF732265 [T]
97 Cortinarius metarius KF732347 [T]
Cortinarius barbarorum DQ663236
Cortinarius flavipallens KF732554 [T]
Cortinarius olympianus KF732364 [T]
74 Cortinarius olympianus KF732553
99 Cortinarius sublilacinopes KF732561 [T]
Cortinarius sublilacinopes DQ663434
Cortinarius pseudogracilior KF732394 [T]
Cortinarius calojanthinus KF732272 [T]
73 Cortinarius corrosus DQ663281
Cortinarius arenicola KF732252 [T]
Cortinarius frondosophilus KF732562 [T]
96 Cortinarius platypus KF732563 [T]
Cortinarius magnivelatus EU056976
Cortinarius spectabilis DQ663425
Cortinarius spectabilis DQ663426
99 Cortinarius sannio KF732536
Cortinarius sannio KF732537
76 Cortinarius pseudoglaucopus DQ663395
97 Cortinarius pseudoglaucopus AY669573
Cortinarius elotoides KF732300 [T]
Cortinarius rapaceoides DQ663417
Cortinarius caroviolaceus EU057049
99 Cortinarius rapaceoides KF732407 [T]
74 Cortinarius rapaceoides NR130253
◆ Cortinarius rapaceoides MN094878
Cortinarius cacodes KF732270 [T]
80 Cortinarius saxamontanus KF732421 [T]
Cortinarius saxamontanus EU057026
Cortinarius cf. aureofulvus KF732544
99 Cortinarius cf. aureofulvus KF732545
Cortinarius cf. aureofulvus KF732543
Cortinarius sulphurinus var. fageticola DQ663439
99 Cortinarius sulphurinus KC842431
Cortinarius sulphurinus DQ663437
Cortinarius luteicolor KF732546
99 Cortinarius luteicolor KF732547
Cortinarius luteicolor FJ717511
Cortinarius citrinipedes KF732281 [T]
Cortinarius osmophorus DQ663368
Cortinarius evosmus KF732302 [T]
99 Cortinarius osmophorus AY174815
Cortinarius cupreorufus KF732548
Cortinarius cupreorufus KF732549
98 Cortinarius cupreorufus KF732550
Cortinarius cupreorufus KF732294 [T]
96 Cortinarius flavobulbus EU057017
Cortinarius flavobulbus KF732305 [T]
98 Cortinarius viridirubescens KF732476 [T]
Cortinarius viridirubescens EU057007
Cortinarius dibaphus DQ663286
79 Cortinarius alnobetulae EU655672
Cortinarius alnobetulae KF732246 [T]
Cortinarius amnicola KF732249 [T]
Cortinarius subpurpureophyllus KF732450 [T]
Cortinarius subpurpureophyllus KF732557
79 Cortinarius napus GU363492
Cortinarius napus KC842428
Cortinarius cf. meinhardii KF732551
96 Cortinarius meinhardii AY174840
Cortinarius parafulmineus EF014269
99 Cortinarius parafulmineus KF732552 [T]
Cortinarius caerulescens AY174863
Cortinarius aureifolius AF268893
0.02

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New records of *Toninia* from China

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Abstract—Four *Toninia* taxa (*T. albilabra, T. poeltii*, *T. tristis* subsp. *Arizonica,* and *T. tristis* subsp. *fujikawae*) are reported for the first time from China. Descriptions with morphological and chemical characters and known distribution are given for each taxon. A key to the species of *Toninia* s.l. in China is also provided.

Keywords—lichen-forming fungi, *Lecanorales*, *Ramalinaceae*, taxonomy

Introduction

Toninia, widely distributed in drought and arid areas, is an important component of desert lichen communities (Timdal 1991, 2002). The current delimitation of the genus is based mainly on the features of the asci and paraphyses (Meijie & al. 2019) and molecular genetic characters (cf. Kistenich & al. 2018, Ekman 2001, Ekman & al. 2008). *Toninia* is characterized by a crustose to squamulose and epruinose to densely pruinose thallus; lecideine, epruinose to densely pruinose apothecia; dark brown to colourless hypothecium; usually colourless, grey, green, or brown epihymenium and exciple that changes colour in K and N; clavate, *Biatora*-type, 8-spored asci; and elliptical to spindle-shaped, colourless, 1–8-celled ascospores. *Toninia*

[#] Congcong Miao & Meijie Sun contributed equally to this work.

was previously placed in *Lecideaceae* (Massalongo 1852; Timdal 1991; Zahlbruckner 1890). With the advent of the molecular age, phylogenetic analysis of lichens is carried out in combination with their morphological and anatomical features; currently, *Toninia* is classified in *Ramalinaceae* (Timdal 2002; Ekman 2001).

In this paper we contribute to the knowledge of *Toninia* in China and add accurate data for the Lichen Flora of China project by reporting on four taxa new to the country: *T. albilabra*, *T. poeltii*, *T. tristis* subsp. *arizonica* and *T. tristis* subsp. *fujikawae*.

Materials & methods

The specimens studied are preserved in the Lichen Section of Botanical Herbarium, Shandong Normal University, Jinan, China (SDNU) and the Lichen Herbarium of the Kunming Institute of Botany, Kunming, China (KUN). The morphological and anatomical characters of the specimens were examined under a COIC XTL7045B2 stereomicroscope and an Olympus CX41 polarizing microscope. Thallus and medulla were tested with K (a 10% aqueous KOH solution), Cl (a solution of aqueous NaOCl), and I (Lugol's iodine) for identification. Lichen substances were identified using standardized thin layer chromatography (TLC) techniques with system C (Orange & al. 2010). Photographs were taken with Olympus SZX16 and BX61 microscope with a DP72 camera.

Taxonomy

Toninia albilabra (Dufour) H. Olivier, Bull. Géogr. Bot. 21: 196 (1911) Fig. 1

Thallus squamulose, indeterminate. Squamules ≤2(–4) mm diam., scattered or contiguous to irregularly imbricate, orbicular or sometimes slightly lobed, weakly concave to weakly convex. Upper side reddish brown, usually epruinose, shiny, with regular and usually deep fissures in the cortex, pores and pseudocyphellae absent. Margin partly to entirely white pruinose. Underside white to pale brown. Algal layer continuous. Lower cortex poorly developed or absent.

Apothecia ≤1.5(–2.5) mm diam., weakly concave to weakly convex, usually persistently (but narrowly) marginate, densely white pruinose or more rarely epruinose. Proper exciple reddish brown throughout or with an additional grey pigment near the rim, lacking crystals. Hypothecium medium brown in upper part, pale brown to colourless in lower part. Hymenium 60–70 µm high; epithecium grey, K+ violet, N+ violet, containing crystals of calcium oxalate. Spores broadly to narrowly fusiform, 1-septate, 13.5–22.5 × 3–4 µm.

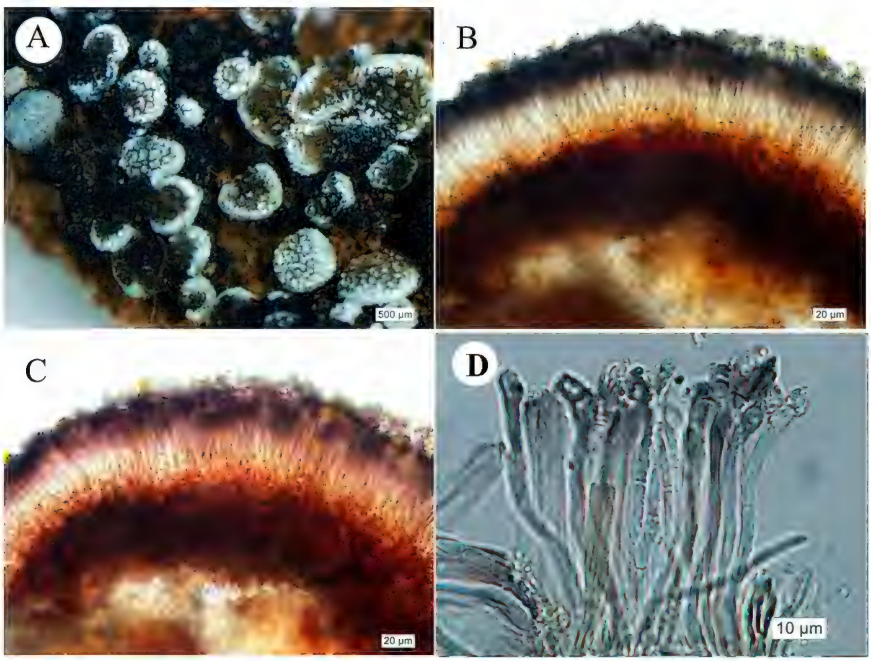

Fig. 1. *Toninia albilabra* (15-49721 KUN).
A. Thallus; B. Epihymenium; C. Purple K reaction of epihymenium; D. Ascospores.

Pycnidia not seen.

Chemistry—Terpenoids detected by TLC.

Specimens examined—**CHINA. Tibet**, **Baju County**, on the way from Bangda to Bangda Airport, alt. 4129 m, 17 Sep. 2014, Wang Lisong & al. 14-47025 (KUN); **Jiangda County**, Tongpu Township, 317 national highway side slope, 31°38′33″N 98°26′10″E, alt. 3940 m, 5 Oct. 2016, Wang Lisong & al. 16-51356 (KUN). **Yunnan**, **Zhongdian**, Benzilan Town, 28°10.47′N 99°23.01′E, alt. 2115 m, 27 Aug. 2006, Wang Li Song & al. 06-26674 (KUN); **Deqin**, Baima Snow Mountain, 28°19.47′N 99°05.13′E, 2 Nov. 2015, alt. 4350 m, Wang Lisong & al. 15-49721 (KUN).

Distribution—On rock or soil. *Toninia albilabra* has been reported in Europe, North Africa, Macaronesia, and the Middle East (Timdal 1991). New to China.

Comments—The Chinese material closely matches the previously published description by Timdal (1991). *Toninia albilabra* is morphologically similar to *T. sedifolia*, which differs mainly by having a smooth upper cortex, a thinner epinecral layer, and farinose pruina. The white edge of the squamules in

T. sedifolia is caused by pruina on the cortex surface and is not sharply delimited from the less pruinose inner part of the upper side. The white edge in *T. albilabra*, however, is caused by the white medulla being visible outside the sharply delimited, usually entirely epruinose, upper cortex (Timdal 1991).

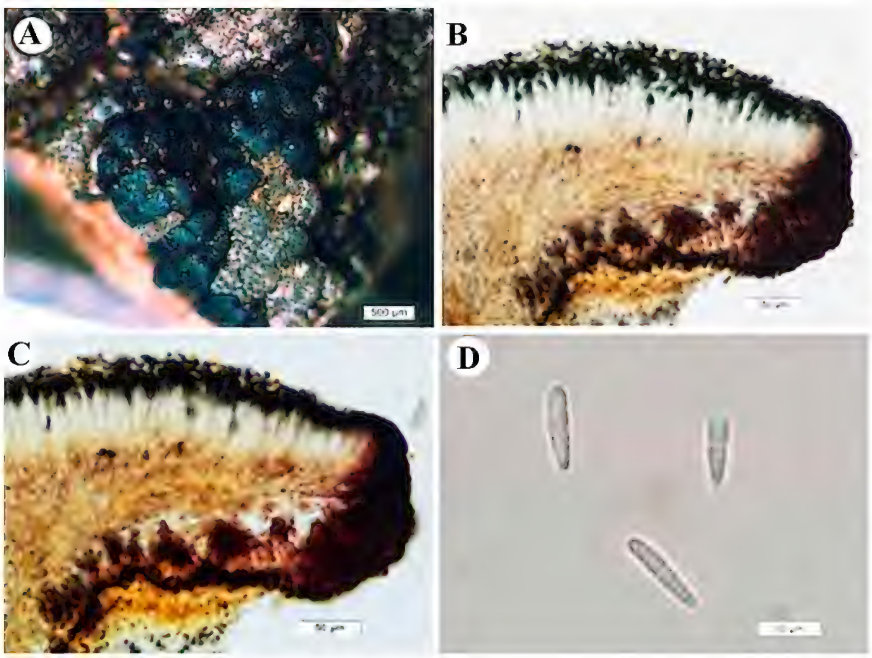

Fig. 2. *Toninia poeltii* (16-53317 KUN).
A. Thallus and apothecia; B. Epihymenium; C. Purple K reaction of epihymenium; D. Ascospores.

Toninia poeltii Timdal, Opera Bot. 110: 85 (1991) Fig. 2

Thallus squamulose, indeterminate. Squamules ≤1 mm diam. More or less continuous crust. Upper side dark greyish brown, epruinose, dull, with a few shallow fissures in the cortex, lacking pores and pseudocyphellae. Margin concolorous with upper side, epruinose. Upper cortex 30–80 µm thick, lacking crystals. Algal layer continuous. Medulla lacking crystals. Lower cortex ≤40 µm thick, brown pigment hyphae.

Apothecia ≤0.8 mm diam., plane and indistinctly marginate when young, later more or less convex and immarginate, epruinose. Proper exciple olivaceous green in the rim, colourless in inner part, K+ violet or brown, N–, lacking crystals. Hypothecium colourless. Hymenium 60–70 µm high;

epithecium olivaceous green, K+ violet or brown, N–. Spores bacilliform, often slightly curved, 1–3-septate, 12–21.5 × 4–5 µm.

Pycnidia not seen.

Chemistry—Terpenoids detected by TLC.

Specimens examined—**CHINA. Tibet**, **Mangkang**, on the way from Lawushan to the town of Rumei, alt. 4290 m, 20 Sep. 2016, Wang Lisong & al. 16-53317 (KUN); **Dingri County**, Alpine meadow, alt. 5250 m, 22 Aug. 2007, Wang Lisong & al. 07-28528 (KUN). **Yunnan**, **Deqin County**, Sola Mountain, 28°38.19′N 98°36.30′E, alt. 4800 m, 10 Sep. 2012, Niu Dongling & al. 12-35865 (KUN).

Distribution—On soil or rock. Reported only in the Himalayas (Timdal 1991). New to China.

Comments—The Chinese material closely matches the previously published description by Timdal (1991). *Toninia poeltii* is morphologically similar to *T. squalida* (which has not been recorded for China), but differs in having an olivaceous green, K+ violet/brown, N– pigment in the epithecium and exciple rim. *Toninia poeltii* has at most 3-septate spores, but *T. squalida* has 3–7-septate spores (Timdal 1991).

Toninia tristis* subsp. *arizonica Timdal, Opera Bot. 110: 112 (1991) Fig. 3

Thallus squamulose, squamules ≤6(–8) mm diam., scattered to adjacent, rounded, bullate but often with an irregular depression. Upper surface castaneous brown to dark brown, epruinose, dull, smooth in the cortex, lacking pseudocyphellae. Upper cortex ≤160 µm high, lacking crystals.

Apothecia ≤6 mm diam., plane and weakly convex, distinctly marginate, epruinose. Proper exciple brown to dark brown, colourless in inner part. Epithecium brown, partly with a green tinge. Spores simple, narrowly ellipsoid to fusiform, 8–17 × 4–4.5 µm. Orange pigment present in the upper part of the hypothecium and in the lumina of many paraphyses, asci, and spores; yellow pigment present in the upper part of the hypothecium.

Chemistry—Terpenoids and fatty acid detected by TLC.

Specimens examined—**CHINA. Inner Mongolia**, **Urad Rear Banner**, Huhe Bash GeTusheng, alt. 1600 m, 19 Aug. 2011, Tong Debao 20123675 (SDNU). **Ningxia**, **Jingyuan County**, Migang Mountain, alt., 2300 m, 20 Jun. 2011, Cheng Yuliang 20116001 (SDNU). **Sichuan**, **Ningnan County**, Baihetan Town, alt. 701 m, 18 Oct. 2013, Wang Lisong & al. 13-39450 (KUN); Jinsha River, alt. 1550 m, 20 Apr. 2014, Wang Lisong & al. 14-43367 (KUN). **Tibet**, **Naqu County**, 317 country road side slope, alt. 4680 m, 30 Sep. 2016, Wang Lisong & al. 16-51961 (KUN). **Yunnan**, **Yunmin County**, Jinsha River Valley, alt. 920 m, 27 Nov. 2014, Wang Lisong & al. 14-46464 (KUN); **Dongchuan**, Sandstone from Dongchuan to Qiaojia Road, alt. 1080 m 11 May 2017, Wang Lisong & al. 17-55053 (KUN).

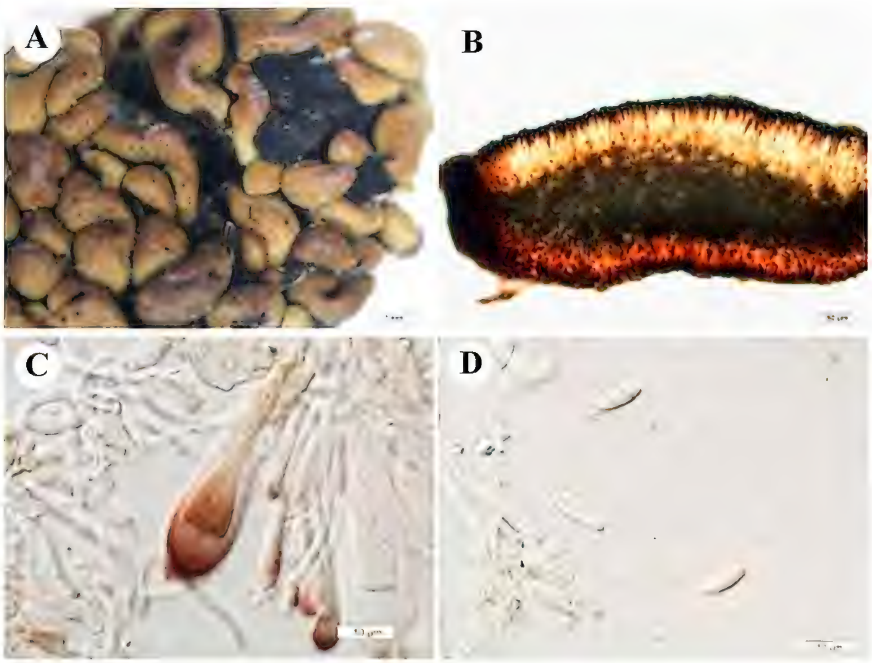

Fig. 3 *Toninia tristis* subsp. *arizonica* (16-53317 KUN).
A. Thallus; B. Epihymenium; C. Ascus; D. Ascospores.

Distribution—On soil. Arizona, USA (Timdal 1991). New to China.

Comments—The Chinese material closely matches the previously published description by Timdal (1991). *Toninia tristis* subsp. *arizonica* produces orange asci similar to *T. tristis* subsp. *asiae-centralis*, which differs in having 1-septate spores (Timdal 1991). In its possession of simple spores, the Chinese material closely matches previously published descriptions of *T. tristis* subsp. *pseudotabacina*, which differs in its absence of orange pigments (Timdal 1991).

Toninia tristis* subsp. *fujikawae (M. Satô) Timdal,
Opera Bot. 110: 113 (1991) Fig. 4

Thallus squamulose, squamules ≤4(–5) mm diam., continuous, bullate, often forming cushions. Upper side castaneous brown to dark brown, lacking pseudocyphellae. Margin concolorous with upper side, epruinose. Upper cortex not containing crystals. Algal layer continuous. Medulla

lacking crystals. Lower cortex resembling upper cortex, but stainable layer often dark reddish brown.

Apothecia ≤4 mm diam., plane and weakly convex, distinctly marginate, epruinose, Proper exciple brown to dark brown, colourless in inner part, K–, N–, lacking crystals. Hypothecium pale brown, 60–70 µm high; epithecium olivaceous green to bright green, K+ violet or brown, N–. Spores simple, ellipsoid, 7–12 × 3–5 µm. Orange and yellow pigments lacking.

Chemistry—Terpenoids and fatty acid detected by TLC.

Specimens examined—**CHINA. Sichuan, Huili County**, Jinsha River, alt. 1550 m, 20 Apr. 2014, Wang Lisong & al. 14-43349; 14-43368 (KUN); **Tibet, Wuqi County**, next to the 214 state road, 31°09′51″N 96°37′54″E, alt. 3840 m, 2 Oct. 2016, Wang Lisong & al. 16-51390 (KUN); **Baqing County**, Laxi village, 317 national road side slope, 31°50′17″N 94°23′24″E, alt. 4180 m, 1 Oct. 2016, Wang Lisong & al. 16-53934 (KUN). **Yunnan, Luquan County**, alt. 2540 m, 19 Apr. 2014, Wang Lisong & al. 14-43234 (KUN); **Deqin County**, Benzilan, 28°11′35.54″N 99°21′08″E, alt. 2112 m,19 Aug. 2018, Wang Chunxiao & al. 20180326 (SDNU).

Distribution—On rock or soil. Japan (Timdal 1991). New to China.

Comments—*Toninia tristis* subsp. *fujikawae* may be confused with *T. tristis* subsp. *canadensis* in having green epithecium, but the latter subspecies has mainly 1-septate ascospores (Timdal 1991). Although also characterized by simple spores, *T. tristis* subsp. *arizonica* is distinguished by a brown epithecium (Timdal 2002).

Key to the species of *Toninia* s.l. in China

Kistenich & al. (2018) place some species below in *Thalloidima* (*T. opuntioides, T. physaroides, T. sedifolia*) or other genera (e.g., *T. gobica*, *T. poeltii*). Here we follow Timdal (1991) and treat all species in *Toninia* s.l.

1. Thallus pruinose (farinose or granular) 2
1. Thallus epruinose to faintly pruinose 14
2. Epithecium gray, K+ violet, N+ violet 3
2. Epithecium brown, K+ red, or epithecium dark green to bright green, K–, N+ violet 13
3. Ascospores at least 1-septate 4
3. Ascospores 0–1-septate ..... *T. toniniana*
4. Ascospores mainly 3-septate 5
4. Ascospores exclusively 1-septate 6
5. Ascospores 1–3-septate, 13.5–22.5 × 3.5–5 µm ..... *T. superioris*
5. Ascospores mainly 3-septate, 23.5–33 × 3–4 µm ..... *T. alutacea*
6. Thallus pruinose, pruina farinose 7
6. Thallus pruinose, pruina granulate 10

7. Thallus rosulate, pruina exposed surface of thallus ..... *T. candida*
7. Thallus not rosulate, pruina patchy on thallus 8
8. Thallus with pseudocyphellae ..... *T. physaroides*
8. Thallus without pseudocyphellae 9
9. Squamules bullate and partly vertically flattened, more or less imbricate, containing an unknown substance (TLC solvent system C Rf. 21) ... *T. opuntioides*
9. Squamules weakly convex to bullate but not vertically flattened or imbricate; not containing an unknown substance (TLC solvent system C Rf. 21) ... *T. sedifolia*
10. Squamules margin usually densely with white pruina ..... *T. albilabra*
10. Pruina not only on the edge of squamules 11
11. Ascospores small (8.5–13 × 4–5 µm) ..... *T. nordlandica*
11. Ascospores larger (12–24 × 3–5 µm) 12
12. Thallus rosulate; apothecia ≤4 mm ..... *T. rosulata*
12. Thallus not rosulate; apothecia ≤1.5 mm ..... *T. diffracta*
13. Epithecium red-brown, K+ red ..... *T. lutosa*
13. Epithecium green, K–, N+ purple; ascospores 3-septate ..... *T. gobica*
14. Thallus epruinose or slightly pruinose 15
14. Thallus epruinose 17
15. Thallus pale brown; epithecium dark brown ..... *T. sculpturata*
15. Thallus of different colour; epithecium not dark brown 16
16. Thallus crustose; epithecium gray, K+ purple, N+ purple ..... *T. pennina*
16. Thallus squamulose, epithecium olive green to bright green, K–, N+ purple; ascospores 1–3-septate ..... *T. aromatica*
17. Epithecium olive green to bright green, K–, N+ purple 18
17. Epithecium red-brown, K+ red; ascospores (1–)7(–9) -septate ..... *T. ruginosa*
18. Thallus crustose; ascospores 1-septate ..... *T. philippea*
18. Thallus squamulose, or crustose to subsquamulose 19
19. Thallus crustose to subsquamulose; epithecium olive green; ascospores 3–7-septate ..... *T. coelestina*
19. Thallus squamulose; epithecium olive green to bright green 20
20. Ascospores simple to 1-septate; epithecium bright green to dark brown .. *T. tristis*
20. Ascospores 1 to more septate; epithecium olivaceous to bright green 21
21. Epithecium olivaceous to bright green (K–), ascospores 1(–3)-septate ..... *T. cinereovirens*
21. Epithecium olivaceous (K+ purple to brown), ascospores 1–(–3)-septate ... *T. poeltii*

Key to the subspecies of *Toninia tristis*

1. Ascus orange, K+ red 2
1. Ascus not orange, K– 3

2. Ascospores 1-septate ..... subsp. *asiae-centralis*
2. Ascospores simple ..... subsp. *arizonica*
3. Epithecium green 4
3. Epithecium brown 7
4. Ascospores 1-septate ..... subsp. *canadensis*
4. Ascospores simple 5
5. Ascospores larger (10–15.5 µm) ..... subsp. *pseudotabacina*
5. Ascospores small (8–12 µm) 6
6. No chemical substance ..... subsp. *thalloedaemiformis*
6. Containing chemical substance ..... subsp. *fujikawae*
7. Ascospores simple ..... subsp. *scholanderi*
7. Ascospores 1-septate 8
8. Squamules ≤2(–3) mm ..... subsp. *tristis*
8. Squamules ≤3(–4) mm ..... subsp. *coahuilae*

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Serendipita sacchari sp. nov. from a sugarcane rhizosphere in southern China

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Abstract—We isolated a new species, proposed here as *Serendipita sacchari*, from a sugarcane rhizosphere in Guangxi Province, China. This species is characterized by its unstable nucleus numbers (1–15) in its chlamydospores versus their regular distribution in hyphal cells. ITS rDNA and combined LSU+ TEF1-α sequence analyses also support the uniqueness of this new plant symbiont.

Key words—molecular phylogeny, *Sebacinales*, *Serendipitaceae*, taxonomy

Introduction

Serendipita P. Roberts (*Basidiomycota, Sebacinales, Serendipitaceae*), typified with *S. vermifera* (Oberw.) P. Roberts, originally comprised seven species (Roberts 1993). Two additional new species *S. lyrica* Trichiès (Trichiès 2003), and *S. herbamans* K. Riess & al. (Riess & al. 2014) have been proposed in this genus, and two anamorphic species in *Piriformospora* Sav. Verma & al. have been recombined as *S. indica* (Sav. Verma & al.) M. Weiss & al., and *S. williamsii* (Zuccaro & M. Weiss) M. Weiss & al. (Verma & al. 1998; Basiewicz & al. 2012; Weiß & al. 2016). *Serendipita* currently contains 11 species and DNA barcodes are widely accepted as an important tool in delineating species (Schoch & al. 2012; Riess & al. 2014).

[#] Ling Xie & Yan-Yan Long contributed equally to this work.

During an investigation of plant endophytes in sugarcane rhizosphere in Guangxi Province, China, an interesting strain has been morphologically and phylogenetically confirmed as a new species of *Serendipita* and is described herein.

Materials & methods

Soil samples were collected from a 5–15-cm sugarcane rhizosphere layer in Tanluo Town, Nanning City, Guangxi Province, China, in Feb 2011. Fungal isolates were obtained by baiting with Chinese cabbage as described by Narisawa & al. (1998), cultured on PDA media, and deposited in the China General Microbiological Culture Collection Center (CGMCC3.19906). A pure culture of the fungus was air-dried and frozen to prepare the holotype specimen, which is curated in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Observations and measurements were made with an Olympus BX53 Ci-L light microscope and Tescan-vega3 LMU scanning electron microscope (SEM).

DNA extraction, amplification, sequencing

Genomic DNA was extracted using the Sangon Biotech Rapid Fungi Genomic DNA Isolation Kit from mycelia grown at 28 °C for 10 d on oatmeal medium (OA, 10 g oatmeal, 10 g bacto agar, 1 g $MgSO_4 \bullet 7H_2O$, 1.5 g H_2PO_4, 1 g $NaNO_3$, for 1 L distilled water). The internal transcribed spacer of ribosomal DNA (ITS rDNA), large subunit of ribosomal DNA (LSU), and translation elongation factor 1-α (TEF1-α) were amplified with fungal specific primer pairs ITS1/ITS4, LROR/LR5, and EF1-983f/EF1-2218r, respectively (Vilgalys & Hester 1990, Basiewicz & al. 2012). PCR reaction mixture and conditions followed the protocol of 2×EasyTaq PCR SuperMix. The DNA was amplified in 50 µL volumes containing PCR buffer [20 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 20 mM Tris–HCl, pH8.4], 200 µM of each dNTP, 15 pmol of each primer, 100 ng DNA template, and 2.5 units of Taq DNA polymerase (Biocolor BioScience & Technology). Thermal cycling began with 5 min of denaturation at 94 °C, followed by a 35 cycles of denaturation (94 °C for 40 s) + annealing (56 °C for 40 s) + extension (72°C for 60 s), and finalized with a final extension at 72 °C for 10 min. A negative control using sterilized distilled water instead of template DNA was included in the amplification process. The PCR products were examined by electrophoresis at 75 V for 2 h in 0.8 % (W/V) agarose gel in 1×TAE buffer (0.4 M Tris, 50 mM NaOAc, 10 mM EDTA, pH 7.8) and visualized under an ultraviolet light after staining with ethidium bromide (0.5 µg mL^{-1}). The PCR products were purified using MultiScreen® PCRµ96 Cleanup Filter Plates according to the manufacturer's protocol. Purified PCR products were directly sequenced with primer pairs mentioned above in an ABI 3730-XL DNA sequencer. The sequences were deposited at GenBank (http://www.ncbi.nlm.nih.gov) and compared through a BLAST search.

Table 1. Taxa with GenBank ITS accession numbers

Species	Voucher	GenBank no.
Cantharellus avellaneus	1217/ER	KX857081
Chaetospermum camelliae	MFLUCC12-0433	KF516965
C. chaetosporum	CBS:154.59[T]	KJ710461
Craterocolla cerasi	TUB 020203	KF061265
Ditangium altaicum	LE 231836 [T]	NR 163760
Efibulobasidium albescens	—	AF384860
Globulisebacina rolleyi	—	AY509550
Helvellosebacina concrescens	TUB 019706	JQ665516
H. helvelloides	TUB 019983	KF000415
Paulisebacina allantoidea	—	AF490396
	RoKi 179	KF061266
Sebacina candida	TUB 020331	KF061278
S. pallida	TUB 019650	JQ665562
Serendipita herbamans	S1[T]	KF061285
S. indica	DSM 11827[T]	KF061284
S. sacchari	**CGMCC3.19906 [T]**	**KY496808**
S. williamsii	DAR 29830	KY509323
Tremellodendron ocreatum	TH8577	KT339265
Tremelloscypha dichroa	VB4212	KF061281

Table 2. Taxa with GenBank LSU and TEF1-α accession numbers

Species	Voucher	GenBank no.	
		LSU	TEF1-α
Sebacina incrustans	TU110840	FJ644513	KF313907
Serendipita. indica	DSMZ 11827[T]	AY505557	AJ249911
S. sacchari	**CGMCC3.19906 [T]**	**KY496809**	**MF196313**
S. vermifera	MAFF 305835	DQ983814	JN211111
	MAFF 305838	DQ983816	JN211116
	MAFF 305828	DQ520096	JN211115
S. williamsii	DAR 29830	AY505556	JN211110

Phylogenetic analyses

The three DNA loci were separately aligned using Clustal-X (1.83). Two neighbour-joining trees were derived from ITS1-5.8S-ITS2 and the combined LSU+ TEF1-α datasets using MEGA v. 4 (Tamura & al. 2007)(Tables 1,2; Figs 2,3).

Bayesian analyses of the aligned DNA sequence dataset were conducted with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) following Sun & Guo (2010). The best-fit evolutionary model was determined for each dataset by comparing different evolutionary models via MrModeltest v. 2.3 (Nylander 2008). Four simultaneous Markov Chain Monte Carlo chains were run by starting from random trees and sampling every 100 generations. The analyses were halted at 4,000,000 generations after the calculation reached stationarity. Of the 40,000 trees generated, 25% were

excluded as "burn in" when calculating the posterior probabilities. Bayesian posterior probabilities were obtained from the remaining 50% majority rule consensus trees. Significant support was defined by clades contained in >95% of the sampled trees.

Taxonomy

Serendipita sacchari L. Xie, Y.Y. Long & Y.L. Chen, **sp. nov.** FIG. 1

MB 836761

Differs from other *Serendipita* species by its variable nucleus numbers (1–15 nuclei) in the chlamydospore cells.

TYPE: China. Guangxi Province: Nanning City, Tanluo Town, in sugarcane rhizosphere, 22°55′30″N 107°58′34″E, alt. 96 m, Apr. 2011 (**holotype**, HMAS 247074; ex-type culture CGMCC3.19906; GenBank KY496808, KY496809, MF196313).

ETYMOLOGY: *sacchari*, referring to the plant from whose rhizosphere the species was first collected.

Colonies reaching 50 mm in diam on PDA medium at 28 °C after 2 weeks, light yellow, circular (or nearly circular), less aerial hyphae. Hyphae hyaline to light yellow-green, septate, 1.2–3.4 μm (2.2 ± 0.5 μm, n = 50), each cell regularly containing 1–2 nuclei. Chlamydospores abundant, spherical to pear-shaped, singly on top of hypha, aseptate. Spores 11.1–17.6 μm (13.5 ± 1.3 μm, n = 55) in diameter, variably containing 1–15 nuclei. Neither conidiophores nor sexual structures observed.

Sequences & the phylogenetic analyses

The obtained ITS (610 bp), LSU (945 bp), and TEF1-α (1131 bp) sequences were deposited in GenBank. To determine the phylogenetic position of *Serendipita sacchari,* all available ITS, LSU, and TEF1-α sequences from *Serendipita* and related genera were downloaded from GenBank (TABLES 1, 2).

The ITS sequences from one isolate of *Serendipita sacchari*, five *Serendipitaceae*, nine *Sebacinaceae*, three *Sebacinales* incertae sedis, and one outgroup *Cantharellus avellaneus*, were included in the phylogenetic analysis. In the alignment of these 19 sequences, the data matrix comprised 591 characters. The alignment dataset was analyzed using MrBayes, applying the GTR+G model selected by MrModeltest as the best-fit model. The prior probability density is a flat Dirichlet (all values = 278 1.0) with both Revmatpr and Statefreqpr as default settings. Posterior probability (BPP) and bootstrap (BS) values are shown on the branches of the ITS Bayesian tree (FIG. 2). The ITS phylogeny places *S. sacchari* in *Serendipitaceae* and in a clade with *S. indica* (BPP = 0.83; NJ support = 97%).

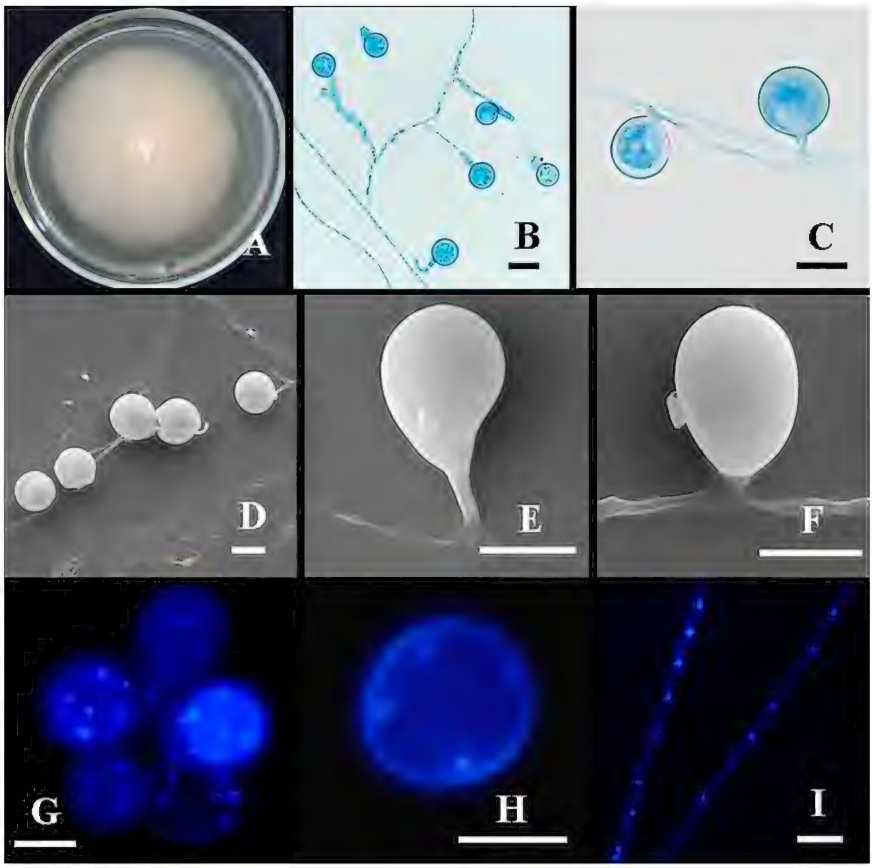

Fig. 1. *Serendipita sacchari* (ex-type, CGMCC3.19906). A: Colony; B, C: Bright field microscopic image of chlamydospores; D–F: Scanning electron microscopic image of chlamydospores; G, H: DAPI–stained chlamydospores; I: DAPI-stained hyphae. Scale bars: B–I = 10 μm.

A combined LSU+TEF1-α dataset comprising one *Serendipita sacchari* isolate, five *Serendipita* isolates, and the outgroup *Sebacina incrustans* was included in a second phylogenetic analysis. These seven sequence pairs (LSU+TEF1-α) aligned in a 1990-character data matrix. The alignment dataset was analyzed in MrBayes, applying the GTR+G model selected by MrModeltest as the best-fit model. The prior probability density is a flat Dirichlet (all values = 278 1.0) with both Revmatpr and Statefreqpr as default settings. Here also BPP and BS values are shown on the branches of the combined Bayesian tree (Fig. 3).

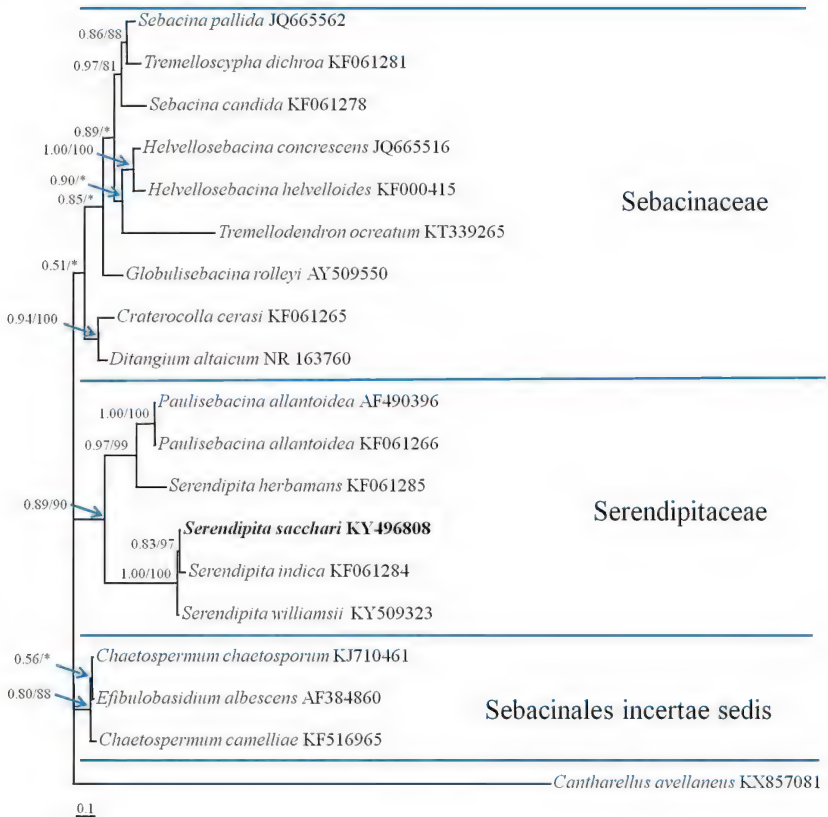

FIG. 2. Phylogenetic tree based on ITS sequences of *Serendipita* and related *Sebacinales* species. *Cantharellus avellaneus* was included as outgroup. The numbers at each branch represented Bayesian posterior probabilities (left) and bootstrap support calculated from 1000 replicates (right). The sequence derived from the proposed new species is in bold. Bar = 0.1 expected changes per site.

In the LSU+TEF1-α phylotree, *S. sacchari* formed a clade with *S. williamsii* with 0.95 BPP support and 100% NJ support. A comparison of the ITS, LSU and, TEF1-α sequence dataset indicates that *S. sacchari* differs from *S. williamsii* in 12/530 bp (2.3%, ITS), 15/930 bp (1.6%, LSU), and 60/1068 bp (5.6%, TEF1-α); and from *S. indica* in 22/552 bp (4.0%, ITS), 21/938 bp (2.2%, LSU), and 63/1132 bp (5.6%, TEF1-α).

COMMENTS. Four species in *Serendipita*—*S. herbamans*, *S. indica*, *S. williamsii*, and *S. vermifera*—were closely related to and allied with *S. sacchari*. Neither conidiophores nor sexual structures were observed in these four taxa. *Serendipita sacchari* can be easily distinguished from *S. herbamans*

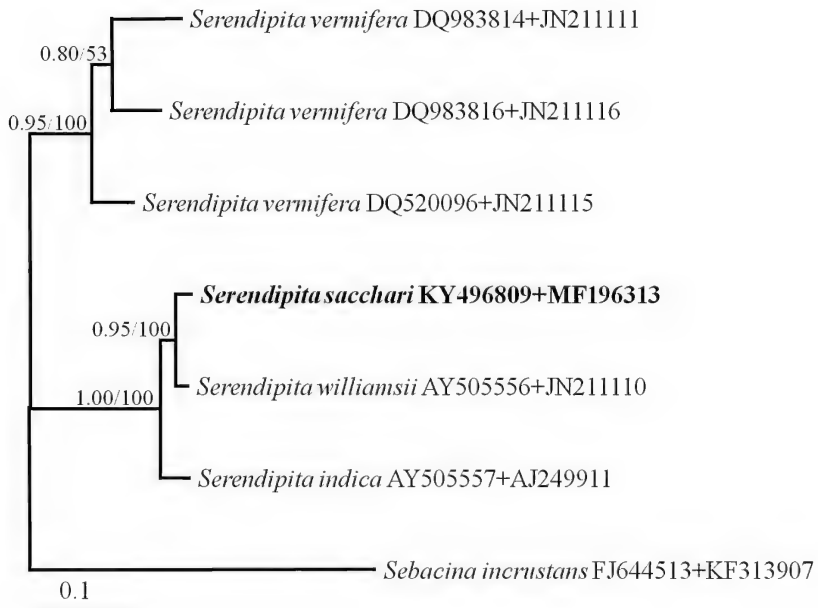

Fig. 3. Phylogenetic tree based on the combined LSU+TEF1-α sequence data of *Serendipita* species. *Sebacina incrustans* was designated as outgroup. The numbers at each branch point represented Bayesian posterior probabilities (left) and bootstrap support calculated from 1000 replicates (right). The sequence derived from the proposed new species is in bold. Bar = 0.1 expected changes per site.

and *S. vermifera* by its bigger (11.1–17.6 µm diam.) chlamydospores (3–5 µm × 4–5 µm in *S. herbamans*; approximately 8 µm diam. in *S. vermifera*; Riess & al. 2014, Ray & Craven 2016). The main difference among *S. sacchari*, *S. indica*, and *S. williamsii* is the distribution and number of nuclei in the cells (Basiewicz & al. 2012). Nuclear number in the chlamydospores of *Serendipita sacchari* (1–15 nuclei) distinguishes the new species from *S. indica* (8–25 nuclei) and *S. williamsii* (≤10 nuclei) (Basiewicz & al. 2012). Moreover, hyphal cells of *S. sacchari* have fewer nuclei and exhibit a more regular distribution compared with *S. williamsii* (2–6 nuclei irregularly distributed) (Verma & al. 1998; Basiewicz & al. 2012). Morphological traits and phylogenetic data both support the recognition of *Serendipita sacchari* as an independent species.

Serendipita herbamans is a common species in plant roots and widely distributed across agricultural and grassland ecosystems. It has the potential to promote the growth of herbaceous plants (Riess & al. 2014). *Serendipita*

indica (≡ *Piriformospora indica*) is widely distributed and well known as a symptomless root endophyte that colonizes bryophytes, pteridophytes, gymnosperms and angiosperms. It has been reported to occur in four continents, is extremely versatile in its mycorrhizal associations, and is known for its ability to promote plant growth (Varma & al. 2012). *Serendipita williamsii* (≡ *Piriformospora williamsii*) was isolated from the spore of arbuscular mycorrhizal fungal in Australia (Basiewicz & al. 2012). *Serendipita vermifera* was isolated from the Australian orchid *Cyrtostylis reniformis* (Warcup 1988). It is reported to induce beneficial effects on plant performance including growth promotion, increase nutrient uptake, enhance seed production, and increase resistance against different biotic and abiotic stresses (Ray & Craven 2016). *Serendipita sacchari* was from sugarcane rhizosphere soil, and symbiotic with Chinese cabbage and sugarcane as a symptomless root endophyte (data not shown). All five *Serendipita* species are plant symbiotic microbionts.

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Five *Nolanea* spp. nov. from Brazil

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Abstract—Five new species of *Nolanea* (*Entolomataceae*, *Agaricales*)—*N. albertinae*, *N. atropapillata*, *N. pallidosalmonea*, *N. parvispora,* and *N. tricholomatoidea*—were collected from the São Paulo metropolitan region. Phylogenetic analyses based on partial sequences of the mitochondrial small subunit (mtSSU), nuclear large subunit (nLSU) and second largest RNA polymerase subunit (rpb2), confirm that all these species belong to *Nolanea*. Morphological descriptions, comments, illustrations, scanning electron microscopy and color photos are included.

Key words—basidiomycetes, *Entoloma*, Neotropical fungi, South America, taxonomy

Introduction

Currently, *Nolanea* has two main circumscriptions, one according to Largent (1994) and Henkel & al. (2014) as *Nolanea* (Fr.) P. Kumm., and the other according to Noordeloos (1992, 2004) and Noordeloos & Gates (2012) as *Entoloma* subg. *Nolanea* (Fr.) Noordel. Both circumscriptions agree that *Nolanea* comprises species often mycenoid with the pileus surface typically hygrophanous, usually translucent-striate and with angled basidiospores with five, six or seven angles in profile view, which can be subisodiametric or heterodiametric. The main difference between the accepted circumscriptions of the genus *Nolanea* is in the pileipellis structure. Largent (1994) considers *Nolanea* to encompass only species that have a pileipellis composed of two layers, with a suprapellis composed of a cutis or a repent entangled hyphae

layer overlying a subpellis of inflated hyphae, whereas Noordeloos accepts species in *Entoloma* subg. *Nolanea* in which the repent, cylindrical hyphae of the pileipellis often (but not necessarily always) overlie a distinct differentiated subpellis of inflated cells.

In general, the few phylogenetic studies that include *Nolanea* species do not define which circumscription is the most appropriate for the group; additionally, they suggest *Nolanea* can be polyphyletic (Co-David & al. 2009, Baroni & Matheny 2011). However, *Nolanea* species are always in the Nolanea–Claudopus clade, independent of the combination of loci used: mtSSU, nLSU, and rpb2 (Co-David & al. 2009, Baroni & Matheny 2011) or ITS, nLSU, and rpb2 (Kinoshita & al. 2012). Also, it is usually possible to visualize two separate branches within the Nolanea–Claudopus clade, with a clade of *Nolanea* species separate from a clade comprising *Claudopus* species.

More recent molecular studies of *Entoloma* subg. *Nolanea* (Vila & al. 2013, Raj & al. 2014, Raj & Manimohan 2016) examined *Nolanea* species using only ITS sequences, with special emphasis on taxa delimitation and new taxa proposition. The phylogeny produced in these studies supports, even with only ITS sequences, the recognition of *Nolanea* as a good phylogenetic group and would seem to support *Nolanea* as an autonomous genus in the *Entolomataceae*.

Nolanea (with about 250 names in MycoBank and Index Fungorum) is considered a common and speciose genus, especially in temperate regions (Noordeloos 1992, 2004, Largent 1994, Horak 2008), but it is also present in tropical and subtropical regions (Romagnesi & Giles 1979). In Brazil of the 23 *Nolanea* species recorded (Rick 1919, 1920, 1930, 1961, Bresadola 1920, Singer 1953, Horak 1977, Capelari 1989, Putzke & Cavalcanti 1997, Pegler 1997, de Meijer 2001, 2006, Rosa 2002), eleven have been cited and/or described by Rick (1919, 1920, 1930, 1961, Singer 1953) and are considered as incertae sedis (Horak 1977). Many other species have been reported in dissertations (Capelari 1989, Rosa 2002), an identification key (Pegler 1997), and lists (Singer 1953, de Meijer 2001, 2006), but only three have been published with a full description (Putzke & Cavalcanti 1997).

In this work, we describe five new species found in the São Paulo metropolitan region accompanied by molecular information and phylogenetic analyses.

Materials & methods

Basidiomata collection and morphological observations

The studied materials were collected mainly at the Parque Estadual da Cantareira and the Parque Estadual das Fontes do Ipiranga in São Paulo City and Reserva

TABLE I. Collections used in the phylogenetic analyses

Cl. = *Claudopus*; *E.* = *Entoloma*; *I.* = *Inocephalus*; *L.* = *Leptonia*; *P.* = *Pouzarella*; *Rh.* = *Rhodocybe*; *R.* = *Richoniella*; *T.* = *Trichopilus*; Sequences generated in this study in **bold.**

NCBI species name	GenBank acc. no.			Voucher	Origin
	RPB2	LSU	mtSSU		
Cl. minutoincanus	HQ731517	HQ731514	HQ731511	DLL 9871	Australia, NSW
Cl. viscosus	HQ731518	HQ731516	HQ731513	DLL 9788	Australia, QLD
E. abortivum	GU384642	GU384616	GU384595	TB6693	USA, NY
	GQ289222	GQ289150	GQ289290	den Bakker 92	Canada
E. albidoquadratum	GQ289223	GQ289151	GQ289291	Manimohan 667-**T**	India, KL
E. bloxamii	GQ289226	GQ289154	GQ289294	Noordeloos 200442	Austria
E. cephalotrichum	GQ289229	GQ289157	GQ289297	Ulje 1997-08-01	Netherlands
E. conferendum	GQ289231	GQ289160	GQ289300	Noordeloos 200313	Belgium
E. cf. *conferendum*	—	JQ320115	—	HKAS 48953	China, SC
E. gasteromycetoides	GQ289235	GQ289164	GQ289304	Gates E2031	Australia, TAS
E. haastii	GQ289238	GQ289167	GQ289307	Noordeloos 2004055	Australia, TAS
E. hebes	GQ289241	GQ289170	GQ289310	Hartman 1992-10-28	Netherlands
E. nidorosum	GU384643	GU384617	GU384596	TB9971	USA, NY
E. pallideradicatum	GQ289247	GQ289176	GQ289316	Hausknecht	Austria
E. parasiticum	GQ289248	GQ289177	GQ289317	Noordeloos 200330	Belgium
E. porphyrescens	GQ289253	GQ289182	GQ289322	Noordeloos 2004113	Australia, TAS
E. procerum	GQ289254	GQ289183	GQ289323	Noordeloos 2004070	Australia, TAS
E. pygmaeopapillatum	GQ289256	GQ289185	GQ289325	Noordeloos 200364	Slovakia
Entoloma readiae	GQ289257	GQ289186	GQ289326	Noordeloos 2004050	Australia, TAS
E. sericatum	GQ289260	GQ289189	GQ289329	Noordeloos 200328	Slovakia
E. sericonitidum	EF421016	AF261315	EF421098	TB7144	USA, NY
E. serrulatum	GQ289263	GQ289192	GQ289332	Noordeloos 2004062	Australia, TAS
E. sinuatum	GQ289264	GQ289193	GQ289333	Wisman 2003-09-19	Netherlands
E. strictius	—	AF042620	—	M96/10	—
E. strictius var. *isabellinus*	GU384641	GU384618	GU384594	TB7710	USA, TN
E. turbidum	GU384656	GU384630	GU384603	TB6949	USA, NY
E. undatum	GQ289270	GQ289202	GQ289342	Noordeloos 200327	Belgium

NCBI species name	GenBank acc. no.			Voucher	Origin
	RPB2	LSU	mtSSU		
E. valdeumbonatum	GQ289271	GQ289203	GQ289343	Meusers E4565 [T]	Germany
I. murrayi	GU384637	GU384620	GU384590	VHAs02.02	—
I. sp. MCA2479	GU384640	GU384622	GU384593	MCA2479	—
L. serrulata	GU384634	GU384624	GU384588	VHAs01.02	—
L. sp. MCA1486	GU384635	GU384623	GU384589	MCA1486	—
N. albertinae	—	**KF738937**	**KF738925**	FK1731	Brazil, RJ
	KF771345	**KF738938**	**KF738926**	FK1732	Brazil, RJ
	KF771344	**KF738936**	**KF738924**	FK0935-**T**	Brazil, SP
N. atropapillata	—	**KF738940**	**KF738929**	FK0898-**T**	Brazil, SP
N. cetrata	**KF771346**	**KF738942**	**KF738927**	DLL9531	USA, CA
	—	AF261319	—	TB7382	USA, NY
N. conferenda	**KF771351**	**KF738946**	**KF738935**	11CA014	USA, CA
N. conferenda	—	AF261321	—	TB7660	USA, NY
N. hirtipes	—	AF261320	—	K1171992	
N. pallidosalmonea	—	**KF738941**	**KF738930**	FK0891	Brazil, SP
N. parvispora	**KF771348**	**KF738943**	**KF738931**	FK1140	Brazil, SC
	—	—	**KF738932**	FK2135	Brazil, SC
N. sericea	DQ367435	DQ367423	EF421099	VHAs 03/02	—
	GQ289262	GQ289191	GQ289331	Noordeloos 200329	Slovakia
	KF771349	**KF738944**	**KF738933**	11CA055	USA, CA
	KF771350	**KF738945**	**KF738934**	11CA056	USA, CA
	—	AF261318	—	TB6506	USA, CA
N. strictior	EF421017	—	EF421100	DUKE-JM96/10	
N. tricholomatoidea	**KF771347**	**KF738939**	**KF738928**	FK1049-**T**	Brazil, SP
P. albostrigosa	HQ876513	HQ876535	HQ876557	Largent 9641	Australia
P. lasia	HQ876507	HQ876529	HQ876555	Largent 9662	Australia
P. nodospora	—	AF261308	—	TB5716	USA, SC
P. setiformis	HQ876503	HQ876525	HQ876525	Largent 9809	Australia
Rhodocybe trachyspora	GU384658	GU384629	GU384605	TB5856	USA, CA
Richoniella asterospora	JF706311	JF706310	—	PBM3268	USA, TN
Trichopilus porphyrophaeus	—	AF261290	—	TB6957	USA, NC
Outgroup					
Clitocybe dealbata	DQ825407	AF223175	AF357138	IE-BSG-HC95cp3	—
Lyophyllum leucophaeatum	DQ367434	AF223202	AF357101	IE-BSG-HAe251.97	—

Biológica de Paranapiacaba in Santo André City, in remnants of the Atlantic Forest in São Paulo State, Southeast Brazil.

Revived sections of tissues from dried basidiomata mounted in 5% KOH, 5% NH_4OH, or 1% aqueous Congo Red dye used to stain hyaline structures were examined microscopically. All the micrographs were drawn with the aid of a drawing tube. 'Q' represents the minimum and maximum length/width quotient, and 'Qm' represents the mean length/width quotient of the total sample of measured spores. The pileipellis structure was examined from the middle portion of the pileus and not the disc or margin. The specimens were deposited in the herbarium of the Instituto de Botânica (SP) and of the Royal Botanic Gardens (K). The samples were prepared for SEM following Baroni (1981) and scanning electron micrographs (SEMs) were made using a LEO 435 VP scanning electron microscope.

DNA extraction, amplification, sequence analyses

The DNA was extracted with hexadecyltrimethylammonium bromide (CTAB) (Ferreira & Grattapaglia 1995) using lyophilized tissues from basidiomata ground to a fine power in liquid nitrogen. The sample was resuspended in 50 µL of TE and stored at –20 °C. The partial sequences of the mitochondrial small subunit (mtSSU) were amplified via the polymerase chain reaction (PCR) primed by MS1 and MS2 (White & al. 1990); the 28S nuclear large subunit (nLSU) rDNA was amplified using LR0R and LR5 (Moncalvo & al. 2000); the ITS1-5.8S-ITS2 was amplified using ITS1-F and ITS4 (White & al. 1990, Gardes & Bruns 1993); and the second largest subunit of the RNA Polymerase II (rpb*2*) using rpb*2*-i6F and rpb2-i7R (Co-David & al. 2009) or rpb2-F1 and rpb2-R1b (Largent & al. 2013). The PCRs were performed in 25–50 µL reaction volumes, where the reaction concentration was 1× for PCR Buffer, 2 mM for MgCl2, 0.2 mM for each dNTP, 0.5 µM for each forward and reverse primer, and 0.025 U of Taq polymerase. Products from multiple reactions were pooled for weak PCR amplifications. The reaction was performed using the following cycling parameters: 94 °C for 2 min, 34 cycles at 94 °C for 45 s, 50 °C (54C° for rpb2) for 1 min and 10 s and 72 °C for 2 min, and then 72 °C for 10 min. PCR amplifications products were purified using the AxyPrep PCR Clean-up Kit (Axygen Biosciences, Union City, USA) or alternatively, using the ExoSapIT kit following the protocols outlined in Largent & al. (2011). DNA sequencing reactions were performed with BigDye Terminator v3.1 Cycle Sequencing Kit. The sequences were run in a ThermoFisher 3730 DNA Analyzer using Sequencing Analysis 5.3.1 and Base Caller KB. The forward and reverse sequences for each sample were aligned and edited using CodonCode Aligner or Sequencher.

The phylogenetic analysis was conducted using sequences obtained in this study and sequences found in GenBank of species traditionally accepted as *Nolanea* [Nolanea–Claudopus clade sensu Co-David & al. (2009) and Baroni & Matheny (2011)] (Table I). *Lyophyllum leucophaeatum* (P. Karst.) P. Karst. and *Clitocybe dealbata* (Sowerby) P. Kummer were selected as outgroups based on phylogenetic analyses performed by Co-David & al. (2009). The sequences were manually aligned using Se-Al Sequence Alignment Editor; spliceosomal introns in the rpb2

were delimited using Augustus ver. 2.4 webserver (Stanke & al. 2008) and omitted along with ambiguous sites in mtSSU. For these phylogenetic analyses, sequences from the three genes including missing data were assembled. The final alignment after the exclusion of introns and ambiguous sites was 3162 bp including gaps: 592 bp for the mtSSU, 1390 bp for the nLSU and 1180 bp for the rpb2 (Tree-BASE database ID14850).

Phylogenetic analyses were performed using maximum likelihood (ML), Bayesian Inference (BI), and maximum parsimony (MP). For the BI, the best-fit model of nucleotide substitution was performed using the Akaike information criterion (AIC) in TOPALI v.2 (Milne & al. 2009). ML analysis was performed using RAxML 7.0.4 with a GTR + G model according to recommendations (Stamatakis 2006, Stamatakis & al. 2008). Support for nodes was tested using 1000 bootstrap replicates. For the BI phylogeny, the best-fit model of nucleotide substitution for each gene partition and codon for the rpb2 (GTR + G for LSU, mtSSU and codons and 3 of rpb2, and GTR + G + I for first codon of rpb2). The BI was performed with MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) specifying four chains (one cold, three heated) for 2,000,000 generations (sampling trees every 1000 generations), with a burn-in of 30,000 generations. MP heuristic searches were performed using the program TNT (Goloboff 1999, Nixon 1999, Goloboff & al. 2008). Tree searches were conducted with equal weights and treating gaps as a fifth character state. Branch support was calculated with 1000 bootstrap replicates. A bootstrap value of 70% was considered significant for ML and MP. The Bayesian posterior probability (BPP) statistical support values of branches were considered informative if ≥ 0.95.

Phylogenetic results

The DNA sequences obtained, including partial sequences of the nLSU, mtSSU, and rpb2m are summarized in Table I. Additionally, sequences obtained from *Nolanea albertinae* (FK0912: KF679349, FK0935 [holotype]: KF679348), *N. atropapillata* (FK0898 [holotype]: KF679354), *N. pallidosalmonea* (FK0891 [holotype]: KF738923), *N. parvispora* (FK1140 [holotype]: KF679353) and *N. tricholomatoidea* (FK1049 [holotype]: KF679352) were deposited in GenBank.

The phylogenetic analyses placed all five species studied into /Nolanea–Claudopus (Co-David & al. 2009, Baroni & Matheny 2011, Kinoshita & al. 2012), an unsupported clade based on ML, MP, and BPP. The phylogenies place all five species into one of two strongly supported internal clades (Fig. 1) in the /Nolanea clade with other species of *Nolanea*.

Taxonomy

Five new species are described and illustrated with line drawings, macroscopic photographs and SEMs of basidiospores.

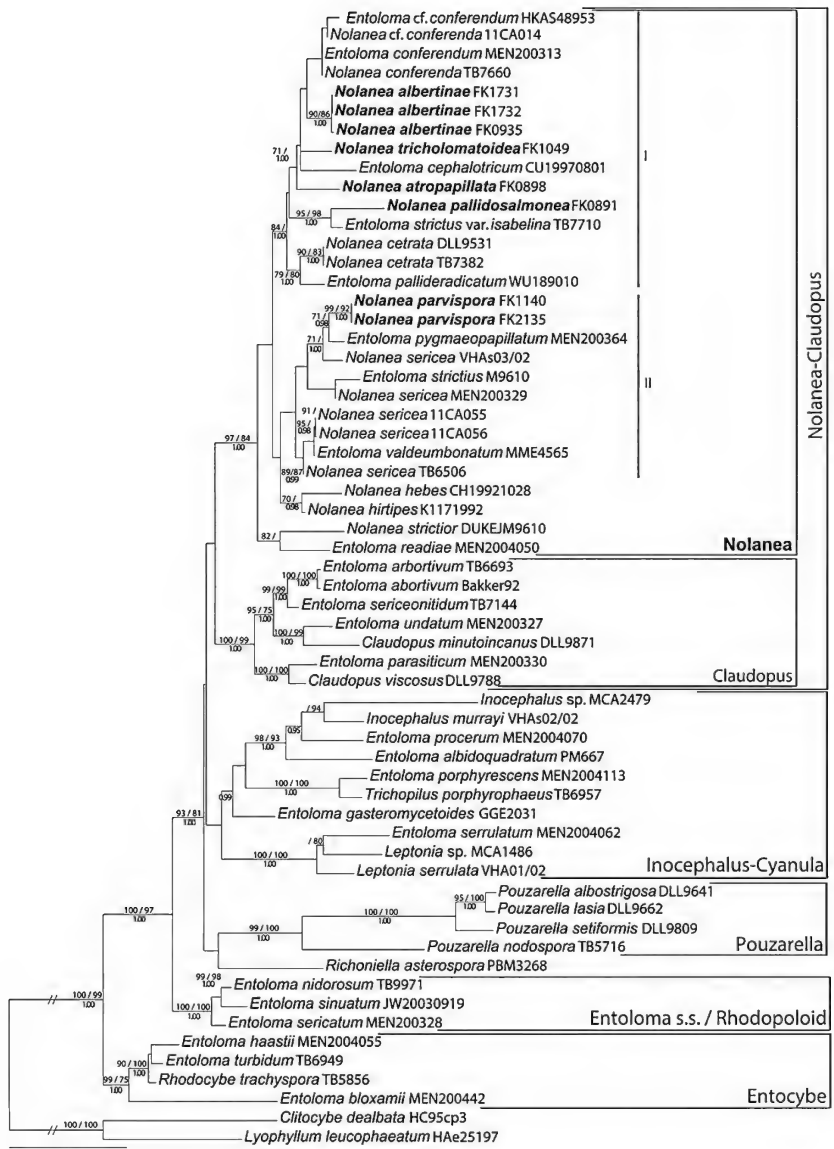

FIG. 1. Phylogram of maximum likelihood (ML) phylogenetic reconstruction using rpb2, nLSU, and mtSSU sequences. Support is indicated with ML/MP bootstrap percentage above and Bayesian posterior probabilities (BPP) below branches. The names of species studied in this paper are in **boldface.** I. Species with heterodiametric basidiospores. II. Species with iso- to subisodiametric basidiospores.

Nolanea albertinae Karstedt & Capelari, **sp. nov.** FIGS. 2A–E, 3, 8A,B

MB826784

Differs from *Entoloma belouvense* by its smaller basidiospores and presence of refractive hyphae.

TYPE—Brazil, São Paulo State, Santo André: Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, trail 15, 18.V.2006, F. Karstedt & M. Capelari FK0935 (**Holotype**: SP; GenBank KF771344, KF738936, KF738924).

ETYMOLOGY—an honour to Albertina Teresinha Karstedt.

PILEUS 14–18 mm diam., broadly conic, conico-convex, convex, plano-convex or plane, often slightly papillate, rarely with the center plane or depressed, pale brown, brown, dark brown (chocolate-colored), or gray-brown, center often darker than the margin, surface glabrous or with a few appressed, concolorous radial fibrils, when humid strongly translucent-striate, when dry, opaque, hygrophanous, margin slightly or strongly eroded. PILEUS CONTEXT very thin, whitish. LAMELLAE adnexed or sinuate, pale pinkish brown, subdistant, with 1–5 lamellulae (3 series), margin slightly eroded. STIPE 36–71 × 1–4 mm, often cylindrical, rarely tapered above or subbulbous over base, brownish beige, pale brown, gray-brown or ocher brown, rarely yellowish brown or concolorous with pileus, apex always paler than the base, sometimes beige or whitish, surface glabrous, often with the apex slightly pruinose, fibrillose-twisted, hollow, base of stipe often with a white tomentum. SPORE PRINT not recorded.

BASIDIOSPORES 7.5–10(–11.2) × 5–7.5 µm (Q = 1.16–1.75, Qm = 1.36), heterodiametric, strongly angled, with 5 or 6 angles in profile, slightly pinkish brown, thin-walled. BASIDIA 23–37 × 8.7–15 µm, clavate, hyaline, thin-walled, tetrasporic. LAMELLAE EDGES heterogeneous. CHEILOCYSTIDIA always present, dispersed or present [in portion of] on the lamellae edges near the stipe, 26–68 × 5–7.5 µm, cylindrical, often apex capitate, hyaline, thin-walled. PLEUROCYSTIDIA absent. LAMELLAR TRAMA subparallel, with hyphae 5–40 µm diam., cylindrical or fusiform, hyaline, thin-walled; subhymenium ramose. PILEITRAMA composed of radially arranged and parallel hyphae, with hyphae 8.7–25 µm diam., inflated or fusiform, hyaline, thin-walled. PILEIPELLIS a cutis, made up of cylindrical hyphae, with brown intracellular pigment, hyphae thin-walled, 3.7–7.5 µm diam., with cylindrical or cylindrical-clavate terminal elements; subpellis made up of inflated or fusiform, hyaline, thin-walled hyphae, 10–37 µm diam. STIPITIPELLIS a cutis of straw yellow, thin-walled hyphae 5–15 µm diam. CAULOCYSTIDIA absent. CLAMP CONNECTIONS present, but inconspicuous. REFRACTIVE HYPHAE present at pileipellis.

FIG. 2. Basidiomata. ***Nolanea albertinae***: a. FK735; b. FK883; c. FK912; d. FK935 (holotype); e. FK1111. ***N. atropapillata***: f. FK649; g. FK898 (holotype). ***N. pallidosalmonea***: h. (FK892, holotype). ***N. parvispora***: i. FK664 (holotype); j. FK689; k. FK794. ***N. tricholomatoidea***: l. (FK1049, holotype). Scale bars = 1 cm. Photos: a, b, d–j, l: F. Karstedt; c, k: M. Capelari.

HABITAT—Solitary or sometimes gregarious, on litter or in soil with fragmented organic material.

ADDITIONAL SPECIMENS EXAMINED—**BRAZIL, SÃO PAULO STATE: São Paulo**, Parque Estadual da Cantareira, Núcleo Engordador, 23°20′S 46°41′W, Macuco trail, 27.IV.2006, F. Karstedt & M. Capelari FK630 (SP); 29.VI.2006, F. Karstedt & M. Capelari FK0690 (SP); 19.IX.2006, F. Karstedt & M. Capelari FK0735 (SP); Cachoeira trail, 20.III.2007, F. Karstedt & al. FK0883 (SP); 29.V.2007, F. Karstedt & al. FK0912 (SP); 13.III.2008, F. Karstedt FK1101 (SP); Parque Estadual das Fontes do Ipiranga, 23°39′S 46°37′W, Nascente trail, 20.VI.2006, F. Karstedt & al. FK0682 (SP); 28.VIII.2008, F. Karstedt & K. Patekoski FK1111 (SP); Santo André, Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, trail 15, 18.V.2006, F. Karstedt & M. Capelari FK0651 (SP), FK0652 (SP); trail 2, 18.V.2006, F. Karstedt & M. Capelari FK0656 (SP); trail 3, 22.VI.2006, F. Karstedt FK0685 (SP); trail 1, 23.VIII.2006, F. Karstedt FK0727

(SP). **Santa Catarina State:** Gaspar, #1111 Pedro Schmitt Street, hill behind the João Pedro Lenfers' house, 02.VIII.2008, F. Karstedt & A.T. Karstedt FK1110 (SP). **Rio de Janeiro State:** Parque Nacional do Itatiaia, 11.I.2011, F. Karstedt, P.B. Schwartsburd & J.B. Pereira FK1731 (SP; GenBank KF738937, KF738925), FK1732 (SP; GenBank KF771345, KF738938, KF738926).

Comments—*Nolanea albertinae* apparently is a common species in the Atlantic Forest of southern and southeastern Brazil; additionally it is usually solitary and almost undetectable in the litter, considering that both have similar color. *Nolanea albertinae* is similar to *N. atripes* Dennis and *N. pseudopapillata* Pegler, which are both described from Central America (Horak 1977, Pegler 1983). It also resembles *Entoloma dissimile* (Singer) E. Horak (described from Argentina; Singer 1969, Horak 1977), *N. fuscifolia* (Peck) Sacc. (described from the United States (Peck 1874, Hesler 1963) but also found in Argentina; Singer 1969) and *Entoloma novum* E. Horak, (described from Chile; Horak 1977). These species have a similar stature including the mycenoid habit; a pileus that is brownish, conical to plane, papillate and hygrophanous; a similar pileipellis with brown pigmented repent hyphae; and similarly shaped and sized basidiospores, 7–10 × 5–7.5 µm. All also can be separated morphologically besides lacking the cylindrical to capitate cheilocystidia that characterize *N. albertinae*.

Nolanea atripes has a darker pileus, no cheilocystidia, and pileipellis with a vacuolar pigment (Pegler 1983). *Nolanea pseudopapillata*, *Entoloma dissimile* and *Entoloma novum* differ (from *N. albertinae*) by the absence of cheilocystidia and clamp connections and by the presence of encrusted pileipellis hyphae (Singer 1969, Horak 1977, Pegler 1983). *Nolanea fuscifolia* grows on wood (instead soil or litter), has a dark brown pileus, lacks cheilocystidia, and has clamp connections; additionally, *N. fuscifolia* was originally described from New York (Peck 1874, Hesler 1963). *Nolanea fuscifolia* sensu Singer (1969) from Argentina, is closer to *Nolanea albertinae* in its very infrequent cheilocystidia and not (or only slightly) encrusted pigmentation; however it is distinguished by its growth on dead wood and by the presence of fusoid-ventricose caulocystidia.

The species most closely related to *Nolanea albertinae* is *Entoloma belouvense* Noordel. & Hauskn. from the Republic of Seychelles, an island archipelago in the Indian Ocean off the east coast of Africa (Noordeloos & Hausknecht 2007). *Entoloma belouvense* has similar macroscopic features and scattered cheilocystidia that are cylindrical and often capitate. However, in addition to its different distribution, *N. albertinae* has distinctly smaller basidiospores (7.5–10 × 5–7.5 µm versus 10-12 x 6-7,5 µm) and the presence of refractive hyphae at pileipellis.

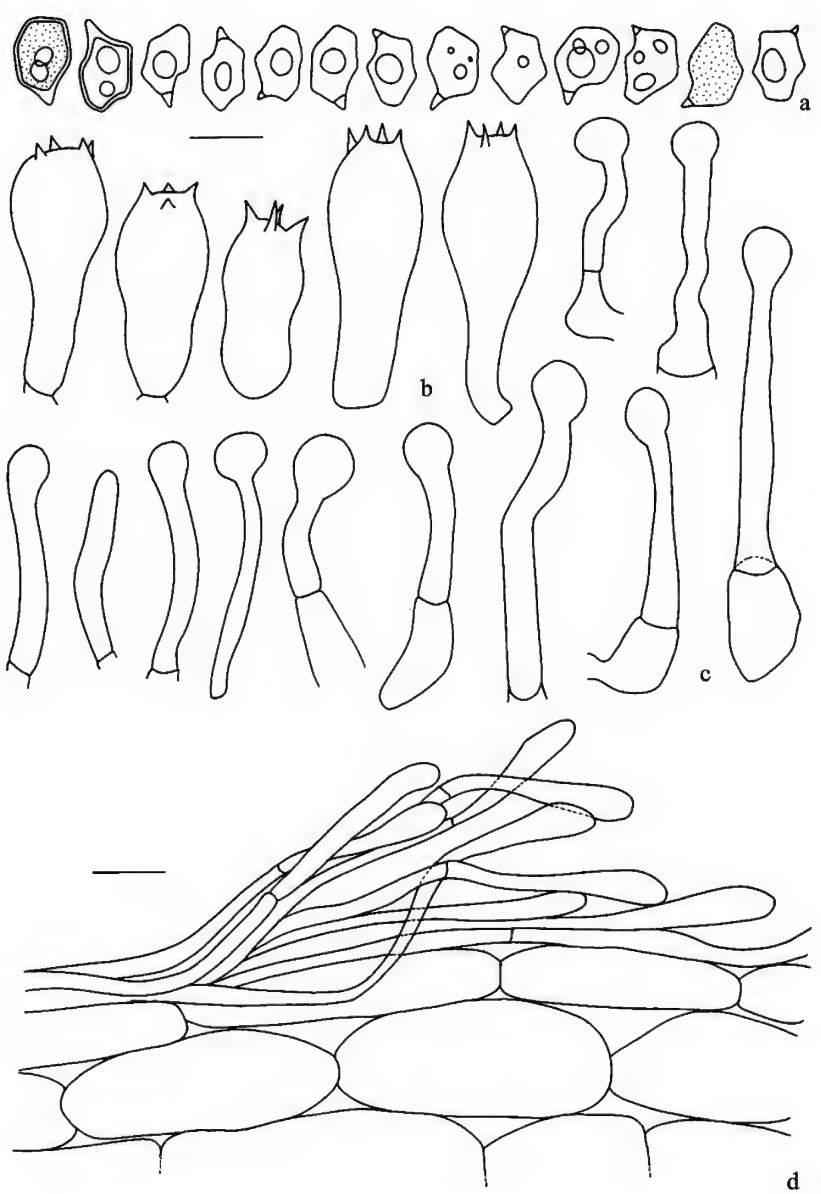

Fig. 3. *Nolanea albertinae* (holotype, FK935):
a. Basidiospores; b. Basidia; c. Cheilocystidia; d. Pileipellis.
Scale bars: a–c = 10 µm; d = 20 µm.

Nolanea atropapillata Karstedt & Capelari, **sp. nov.** FIGS. 2F,G, 4, 8C,D

MB805603

Differs from *N. albertinae, N. atripes, N. pseudopapillata*, and *Entoloma dissimile* by its bisporic basidia, by its intracellular and encrusted pigment in pileipellis hyphae, and by the absence of clamp connections.

TYPE—Brazil, São Paulo State, Santo André: Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, Estrada Frederico Carlos Hoehne, 12.IV.2007, F. Karstedt & al. FK0898 (**Holotype**: SP, GenBank KF738940, KF738929).

ETYMOLOGY—refers to the color of the papillae that are darker than the pileus.

PILEUS 9–25 mm diam., convex, plano-convex or plane, papillate or umbonate, brown or dark brown, darker in the center compared to the edge, turning grayish brown or pale gray-brown with blackish papilla, surface smooth or sometimes slightly squamulose-fibrillose at center, striate-translucent, strongly hygrophanous then silky, margin slightly eroded or split and upturned with age. PILEUS CONTEXT very thin. LAMELLAE almost free, narrowly adnexed, pink or pink-brown, subdistant, with 1–5 lamellulae (2 tiers), margin smooth or slightly eroded. STIPE 20–55 × 1–7 mm, cylindrical, with subbulbous base or slightly tapering upwards from swollen base, nearly white, pale beige or very pale brown, apex that is paler than the base, fibrillose-twisted, hollow, base of stipe often with a white tomentum. SPORE PRINT not recorded.

BASIDIOSPORES 8.7–12.5 × 6.2–8.7 µm (Q = 1.16–1.6, Qm = 1.4), heterodiametric, strongly angled, with 5–6 angles in profile, slightly brownish pink, thin-walled. BASIDIA 20–35 × 7.5–10 µm, clavate, hyaline, thin-walled, bisporic. LAMELLAE EDGE fertile. CHEILOCYSTIDIA and PLEUROCYSTIDIA absent. LAMELLAR TRAMA made up of regular cylindrical or fusiform, hyaline, thin-walled hyphae, 6.2–26 µm diam.; subhymenium ramose. PILEITRAMA composed of radially arranged and parallel cylindrical or fusiform, hyaline, thin-walled hyphae, 3.7–37 µm diam. PILEIPELLIS a cutis, suprapellis made up of cylindrical hyphae, with brown intracellular pigment, sometimes with brown encrusted pigment, hyphae thick or thin-walled, 2.5–18 µm diam.; terminal elements repent or anticlinal and then almost a trichoderm, cylindrical or cylindrical-clavate at center of pileus; subpellis composed of inflated or fusiform, hyaline or slightly straw yellow, thin-walled hyphae, 13.7–44 µm diam. STIPITIPELLIS a cutis made up of hyaline or slightly straw yellow, thin-walled hyphae, 2.5–10 µm diam. CAULOCYSTIDIA absent. CLAMP CONNECTIONS absent. REFRACTIVE HYPHAE not observed.

HABITAT—Solitary or dispersed in litter.

ADDITIONAL SPECIMENS EXAMINED—**BRAZIL, São Paulo State, Santo André**: Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, trail 12, 18.V.2006, F. Karstedt

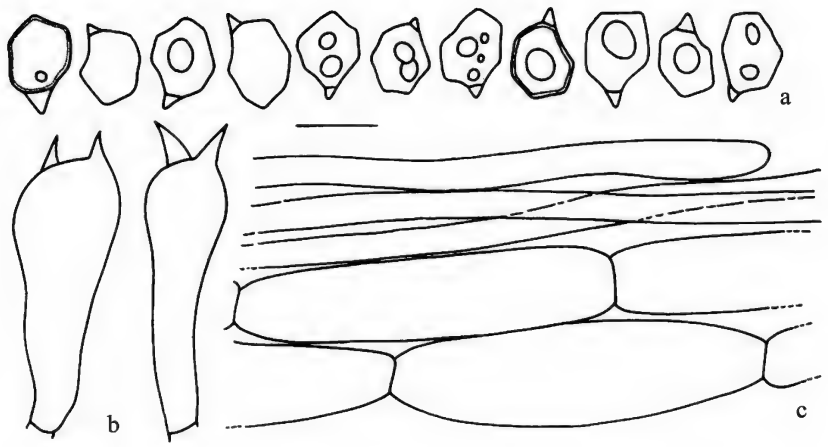

FIG. 4. *Nolanea atropapillata* (holotype, FK898): a. Basidiospores; b. Basidia; c. Pileipellis. Scale bars: a, b = 10 µm; c = 20 µm.

& M. Capelari FK0649 (SP), FK0650 (SP); trail 6, 18.V.2006, F. Karstedt & M. Capelari FK0655 (SP); Estrada Frederico Carlos Hoehne, 12.V.2007, F. Karstedt & al. FK0899 (SP).

COMMENTS—*Nolanea atropapillata* resembles *N. albertinae*, *N. atripes*, *N. pseudopapillata*, and *Entoloma dissimile* from South America and *N. fuscifolia* from the United States. All of these species have papillate, pale brown pilei with a darker center and a translucent-striate surface and heterodiametric basidiospores in the 8–13 × 6–9 µm range. However, all differ from *N. atropapillata* in producing tetrasporic basidia and clamp connections. *Nolanea atripes* is further distinguished by its slender basidioma, membranaceous pileus, and black stipe (Dennis 1961). The description of *N. atripes* by Pegler (1983) differs from the Dennis's type description, in which Dennis (1961) emphasized the contrast between the black stipe and gray-brown pileus, while Pegler (1983) described the stipe as pale brown to dark, and the basidiomata illustrated is much more robust.

Nolanea albertinae also differs by the presence of cheilocystidia and absence of encrustations in the pileipellis hyphae, and *Entoloma dissimile* differs by the pileipellis as a cutis without a differentiated subpellis. *Nolanea fuscifolia* is distinguished by its subnodulose basidiospores (Hesler 1963, Singer 1969) and *N. pseudopapillata* by its strongly heterodiametric basidiospores (Q = 1.61, Pegler 1983).

Nolanea pallidosalmonea Karstedt & Capelari, **sp. nov.** FIGS. 2H, 5, 8E,F

MB805604

Nolanea pallidosalmonea differs from other *Nolanea* spp. by its delicate and slender basidiomata, by a pale pinkish salmon pileus with salmon pink striations and slightly brownish center, by 5–6-angled heterodiametric basidiospores, by a pileipellis composed of repent hyphae, and by the presence of refractive hyphae.

TYPE—Brazil, São Paulo State, Santo André: Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, trail 1, 23.III.2007, F. Karstedt & al. FK0892 (**Holotype**, SP; **isotype** K).

ETYMOLOGY—the name refers to the pale salmon pink coloration of the basidioma.

PILEUS 4–15 mm diam., convex, slightly papillate, when young with more pronounced papillae, very pale salmon pink or whitish salmon pink, darker at the center and strongly translucent-striate when in fresh condition, hygrophanous and then whitish from the margin towards the center and finally whitish salmon pink and when dry, sometimes slightly brownish at center, translucent-striate, after opaque and silky-fibrillose, margin entire. PILEUS CONTEXT thin, translucent. LAMELLAE adnexed or sinuate, pale pink, ventricose, subdistant, with 2 tiers of lamellulae. STIPE 25–54 × 1–3 mm, cylindrical or slightly tapering, pale salmon pink or light brownish, hollow, fibrous, with a small amount of white tomentum at the base. SPORE PRINT not recorded.

BASIDIOSPORES 8.7–10 × 6.2–7.5 µm (Q = 1.16–1.6; Qm = 1.41), heterodiametric, strongly angled, with (5)6–7 angles in profile view, hyaline or slightly pinkish brown, thin-walled. BASIDIA 25–37 × 8.7–11.2 µm, clavate, hyaline, thin-walled, tetrasporic. PLEUROCYSTIDIA and CHEILOCYSTIDIA absent. LAMELLAE EDGE fertile. LAMELLAR TRAMA subparallel, hyphae with 5–20 µm diam., cylindrical or slightly inflated, sometimes fusiform, hyaline, thin-walled; subhymenium ramose. PILEITRAMA trama composed of regular cylindrical, inflated or fusiform, hyaline, thin-walled hyphae, 11.2–26 µm diam. PILEIPELLIS a cutis made up of cylindrical, hyaline, thin-walled hyphae, 2.5–8.7 µm diam.; subpellis made up of inflated or fusiform, hyaline, thin-walled hyphae, 10–21 µm diam. STIPITIPELLIS a cutis of hyaline, thin-walled hyphae, 3.7–12.5 µm diam. CAULOCYSTIDIA not observed. CLAMP CONNECTIONS absent. REFRACTIVE HYPHAE abundant in pileipellis.

HABITAT— Gregarious or scattered, on soil.

ADDITIONAL SPECIMEN EXAMINED—**BRAZIL, SÃO PAULO STATE, Santo André**: Reserva Biológica de Paranapiacaba, 23°46′S 46°18′W, trail 1, 23.III.2007, F. Karstedt & al. FK0891 (SP; GenBank KF738941, KF738930).

COMMENTS—*Nolanea pallidosalmonea* resembles *Entoloma quadratum* (Berk. & M.A. Curtis) E. Horak, cited from Borneo, Singapore, Costa Rica, United

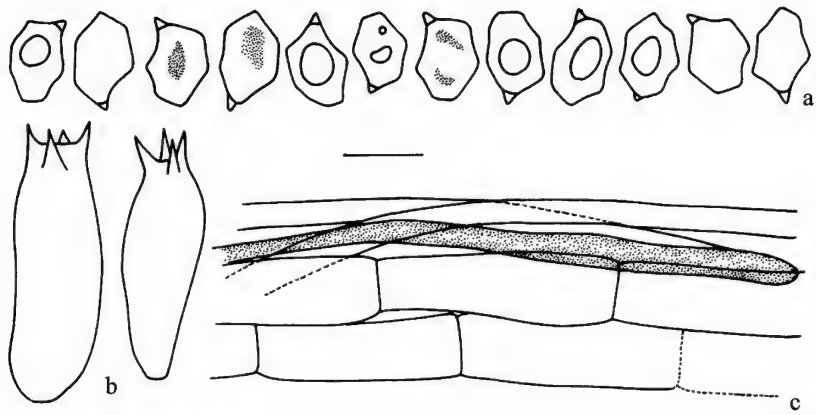

Fig. 5. *Nolanea pallidosalmonea* (holotype, FK892): a. Basidiospores; b. Basidia; c. Pileipellis. Scale bars: a, b = 10 µm; c = 20 µm.

States, Madagascar, Malaysia and Papua New Guinea, in its conical pileus and a smooth surface (a cutis) that is salmon colored. However, *E. quadratum* is clearly separated from *N. pallidosalmonea* by its cuboidal basidiospores and clavate cheilocystidia (Horak 1975, 1977, Baroni & Halling 2000).

Nolanea minuta P. Karst., described from Europe, is another species with pale and pinkish basidiomata, a silky-fibrillose pileus surface, heterodiametric basidiospores, and absence of cheilocystidia. However, *N. minuta* differs from *N. pallidosalmonea* in basidiospores with 5–7 angles in profile view, and a pileitrama composed of hyphae with intracellular and encrusted pigments (Noordeloos 1980).

Nolanea parvispora Karstedt & Capelari, **sp. nov.** Figs. 2I–K, 6, 8G–I

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Nolanea parvispora differs from other *Nolanea* spp. by its lignicolous habitat, pale beige pileus with dark center, stipe with a dark base and pale apex, sinuate lamellae, small (6.2–7.5 × 6.2–7.5 µm) isodiametric basidiospores, a lack of cystidia, and a pileipellis composed of repent hyphae with dispersed groups of anticlinal hyphae.

Type—Brazil, São Paulo State, Santo André: Parque Estadual da Cantareira, 23°20′S 46°41′W, Núcleo Engordador, Cachoeira trail, 25.V.2006, F. Karstedt & M. Capelari FK0664 (**Holotype**, SP).

Etymology—refers to the small size of the basidiospores.

Pileus 13–23 mm diam., convex, plano-convex or plane, sometimes with a slightly or prominent papilla or slightly depressed at the center, whitish beige

or pale brownish beige or light pinkish beige, brown at the center (insertion of stipe), smooth and sometimes pruinose at the center, glabrous, translucent-striate, margin revolute, even, or sometimes lobate. Pileus context very thin. Lamellae sinuate, white or pinkish beige, abundant, with 5–7 lamellulae (2 series), margin even. Stipe 25–42 × 2–4 cm, cylindrical or flattened, brown or brownish translucent, beige at the apex, surface smooth, base with white tomentum. Spore print pinkish. Basidiospores 6.2–7.5 × 6.2–7.5 µm (Q = 1–1.2; Qm = 1.1), isodiametric, with 5–6 angles in profile view, slightly pinkish, thin-walled. Basidia 27–43 × 8.7–10 µm, clavate, hyaline, thin-walled, tetrasporic. Pleurocystidia and cheilocystidia absent. Lamellae edge fertile. Lamellar trama subregular, cylindrical, inflated or fusiform, hyaline, thin-walled hyphae 3.7–12.5 µm diam.; subhymenium ramose. Pileitrama regular, made up of cylindrical, inflated or fusiform, hyaline, thin-walled hyphae, 3.7–25 µm diam. Pileipellis a cutis composed of cylindrical, hyaline or pale straw yellow, thin-walled hyphae, 6.2–13.7 µm diam., with dispersed or clustered thin-walled cylindrical or clavate, hyaline or pale straw yellow anticlinal terminal elements; subpellis made up of inflated or fusiform, hyaline, thin-walled hyphae 10–20 µm diam. Stipitipellis a cutis, made up of hyaline or pale straw yellow, sometimes with encrusted (external) yellow-brown pigment, thin or thick-walled hyphae, 6.2–16.2 µm diam. Caulocystidia rare, cylindrical, clavate or ventricose, with pale straw yellow plasmatic pigment, thin-walled, 28–75 × 2.5–7.5 µm. Clamp connections absent. Refractive hyphae present in pileipellis.

Habitat—Solitary or gregarious, on hardwood.

Additional specimens examined—**BRAZIL, São Paulo State, São Paulo**: Parque Estadual da Cantareira, Núcleo Engordador, 23°20′S 46°41′W, Macuco trail, 29.VI.2006, F. Karstedt & M. Capelari FK689 (SP); Cachoeira trail, 29.VI.2006, F. Karstedt & M. Capelari FK0696 (SP); 25.VII.2006, F. Karstedt & M. Capelari FK0723 (SP); 24.X.2006, F. Karstedt & M. Capelari FK794 (SP); 30.X.2007, F. Karstedt & al. FK0926 (SP). **Santa Catarina State**, Joinville: Reserva Particular de Patrimônio Natural Caetezal, 02.II.2009, F. Karstedt FK1140 (SP; GenBank KF771348, KF738943, KF738931); Corupá, Reserva Particular de Patrimônio Natural Emíleo F. Battistella, falls route, 12.XII.2011, F. Karstedt FK2135 (FLOR; GenBank KF738932).

Comments—*Rhodophyllus avellanicolor* Romagn. & Gilles, *Nolanea mazophora* (Berk. & Broome) Pegler, *Entoloma myceliosum* E. Horak, and *Entoloma imbecille* (E. Horak) E. Horak ex Segedin & Pennycook [≡ *E. fragile* E. Horak, nom. illegit.] are among the few species that resemble *Nolanea parvispora* based on pale basidiomata and isodiametric basidiospores measuring c. 7 × 7 µm. *Rhodophyllus avellanicolor*, described from Africa, and

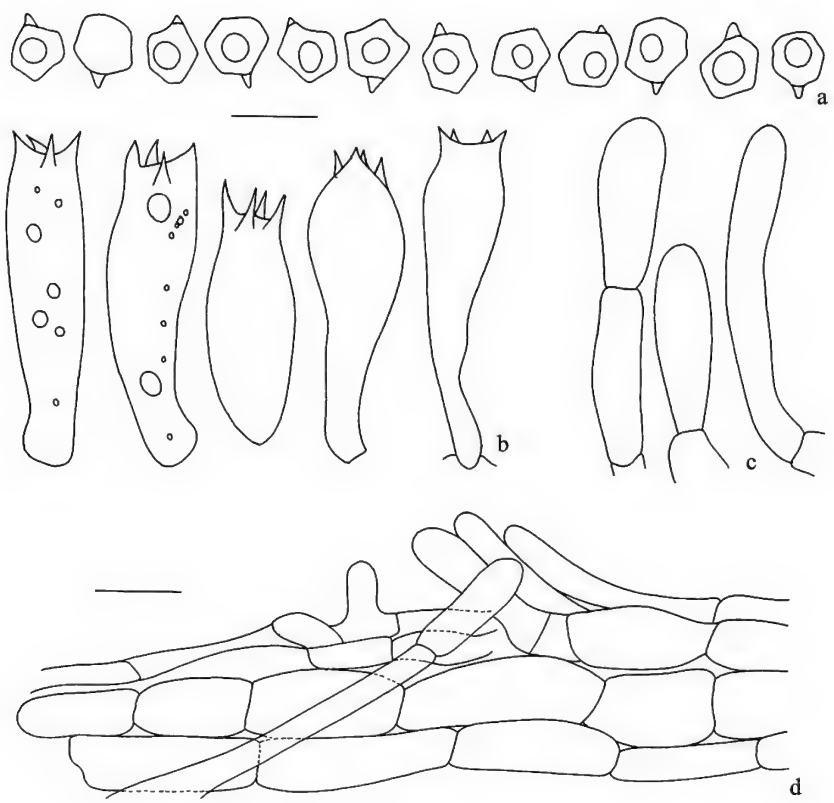

Fig. 6. *Nolanea parvispora* (holotype, FK664):
a. Basidiospores; b. Basidia; c. Caulocystidia; d. Pileipellis.
Scale bars: a–c = 10 µm; d = 20 µm.

Nolanea mazophora, described from Sri Lanka, are terrestrial. *Rhodophyllus avellanicolor* has clamp connections (Romagnesi & Gilles 1979) and *N. mazophora* has a convex pileus with prominent papillae (Pegler 1977, Horak 1980).

Entoloma myceliosum, described from Chile, is also lignicolous but differs from *N. parvispora* by having a white pileus and stipe and a pileus surface densely covered by white fibrils (Horak 1977). *Entoloma imbecille,* found in New Zealand (Horak 1973, as "*E. fragile*") and Argentina (Horak 1977, as "*E. fragile*"), differs by a pileus that is initially brown and then turns beige, brown encrusted pigments in the pileipellis hyphae, and abundant clamp connections.

Nolanea tricholomatoidea Karstedt & Capelari, **sp. nov.** FIGS. 2L, 7, 8J,K

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Nolanea tricholomatoidea differs from other *Nolanea* spp by its robust stature, a mostly beige pileus that turns brownish beige at the center; its whitish beige stipe, its often pentagonal basidiospores; its basidial basal clamp connections at the base of the basidia; and the encrusted pigment in the subpellis hyphae.

TYPE—Brazil, São Paulo State, Santo André: Parque Estadual da Cantareira, Núcleo Engordador, 23°20′S 46°41′W, Cachoeira trail, 31.I.2008, F. Karstedt, L.A. Silva Ramos & M. Capelari FK1049 (**Holotype**, SP; GenBank KF771347, KF738939, KF738928).

ETYMOLOGY—the name refers to the tricholomatoid stature.

PILEUS 80–92 mm diam., conico-campanulate or plane, umbonate, pale beige, beige or brownish beige at center, surface smooth or slightly pruinose at center, opaque, hygrophanous, translucent-striate at margin. PILEUS CONTEXT white. LAMELLAE adnexed (almost free), beige, ventricose, subdistant, with 2 tiers of lamellulae. STIPE 100–116 × 8–12 mm, and 39 mm at base, subclavate, beige or whitish, fibrous and fibrillose, with white basal mycelium. ODOR indistinct. SPORE PRINT not recorded.

BASIDIOSPORES 8.7–10 × 7.5–8.7 µm (Q = 1–1.33; Qm = 1.2), isodiametric or subisodiametric, strongly angled, with 5–6 angles in profile view, with an obvious hilar appendix, hyaline or slightly brownish pink, thin-walled. BASIDIA 37–51 × (8.7–)10–15 µm, clavate with a narrow base, hyaline, thin-walled, tetrasporic. LAMELLAE EDGE fertile. PLEUROCYSTIDIA and CHEILOCYSTIDIA absent. LAMELLAR TRAMA regular and hyaline, with hyphae 3.7–17.5 µm diam., cylindrical or slightly inflated, hyaline, thin-walled, septate; subhymenium ramose. PILEITRAMA regular, composed of cylindrical or inflated, hyaline, thin-walled hyphae 3.7–27 µm diam. PILEIPELLIS a cutis, made up of cylindrical, hyaline, thin-walled hyphae 2.5–8.7 µm diam., with rare anticlinal elements; subpellis composed of inflated, hyaline, thin-walled hyphae 16.2–46 × 50–120 µm diam. STIPITIPELLIS a cutis made up of straw-yellow, hyaline, thin-walled hyphae, 3.7–7.5 µm diam. CAULOCYSTIDIA absent. CLAMP CONNECTIONS present in the hymenium. REFRACTIVE HYPHAE present in the pileipellis.

HABITAT—Solitary, on soil.

ADDITIONAL SPECIMEN EXAMINED—**BRAZIL. RIO DE JANEIRO STATE, Nova Iguaçu**: Reserva Biológica de Tinguá, Ouro road, 8 Dec 2004, M.P. Albuquerque & A.A. Carvalho-Jr. 29-VI (RB415680).

COMMENTS—*Nolanea tricholomatoidea* was collected in Rio de Janeiro State but reported as *Entoloma lividoalbum* (Kühner & Romagn.) Kubička

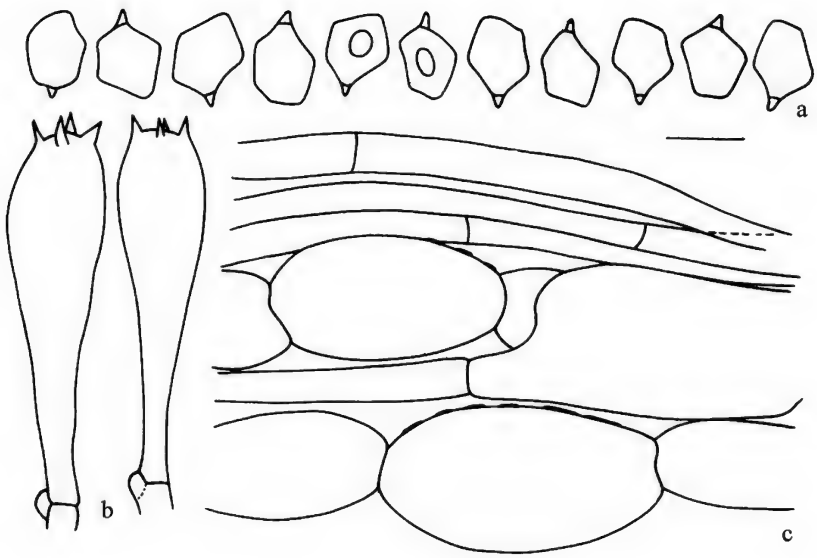

Fig. 7. *Nolanea tricholomatoidea* (holotype, FK1049):
a. Basidiospores; b. Basidia; c. Pileipellis.
Scale bars: a, b = 10 µm, c = 20 µm.

(Albuquerque & al. 2007). The robust stature is reminiscent of a feature common to *Entoloma* sensu stricto, and based mainly on its stature, its umbonate pileus that is sometimes translucent-striate, and its white stipe, *N. tricholomatoidea* might seem related to species of *Entoloma* s.s., such as *E. lividoalbum* and *E. prunuloides* (Fr.) Quél.

Entoloma lividoalbum, described from Europe and recorded from the United States, differs in its pileus that varies from dark brown, dark yellowish brown, pale yellowish white to pale grayish beige, and clamp connections that are present in all tissues (Largent 1994, Noordeloos & Polemis 2008). *Entoloma prunuloides*, recorded from Europe, differs in its smaller basidiospores (6.5–8 × 6.5–8 µm), an ixocutis-type pileipellis, and the presence of clamp connections in all tissues (Noordeloos 1992).

Although a tricholomatoid stature is uncommon in *Nolanea*, the pileipellis structure in *N. tricholomatoidea,* comprising a subpellis with inflated elements and a suprapellis with thin elements, is a feature shared among many *Nolanea* spp.

Discussion

The phylogenetic analyses as supported by ML, MP, and BPP cluster the species described here and other species traditionally accepted as *Nolanea* within a single clade, the /Nolanea clade (Fig. 1).

Four of the species described in this paper—*Nolanea albertinae*, *N. atropapillata*, *N. pallidosalmonea,* and *N. tricholomatoidea*—occur in litter or soil and have heterodiametric basidiospores. They cluster in clade I. *Nolanea parvispora,* the only lignicolous species and characterized by isodiametric basidiospores, clusters in clade II.

Species that are placed in *Nolanea* often have a mycenoid habit (Henkel & al. 2014, Largent 1994, Noordeloos 1992, 2004, Noordeloos & Gates 2012). *Nolanea tricholomatoidea* described herein and the two species classified as *Entoloma* s.s. (*E. pallideradicatum* Hauskn. & Noordel. and *E. valdeumbonatum* Noordel. & Meusers) have a tricholomatoid habit and yet are included in the /Nolanea clade. These species share with *Nolanea* species a pileipellis with a subpellis composed of inflated hyphae and heterodiametric basidiospores.

Curiously, all species studied and almost all species (that have been published with a detailed description of the pileipellis) in the /Nolanea clade have a pileipellis composed of a suprapellis with narrow hyphae and a subpellis with inflated hyphae. We recommend paying careful or close attention to this character in the future to determine if these features are phylogenetically informative.

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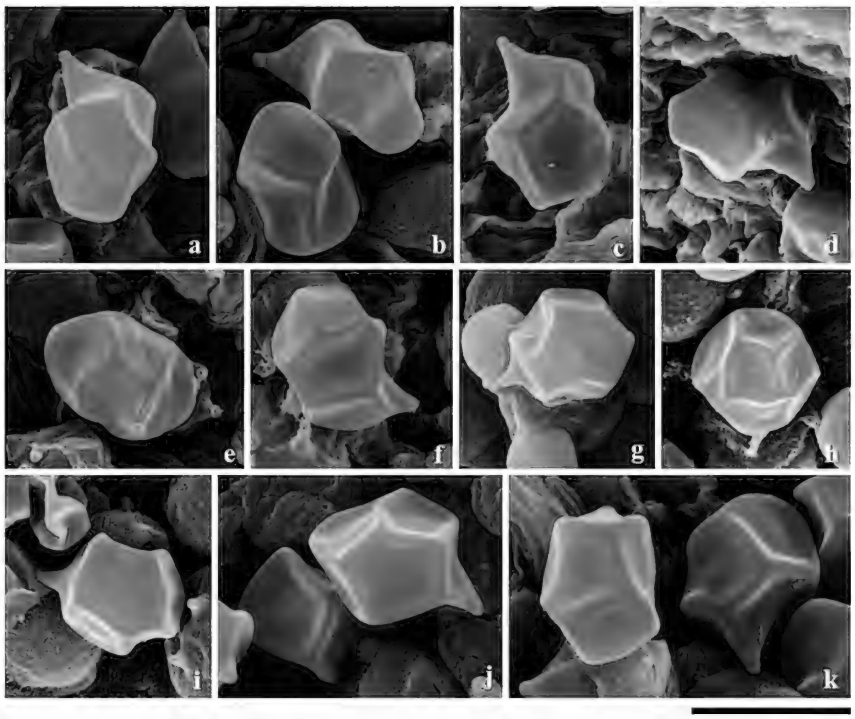

Fig. 8. Basidiospores in SEM:
a, b. *Nolanea albertinae*; c, d. *N. atropapillata*;
e, f. *N. pallidosalmonea*; g–i. *N. parvispora*;
j, k. *N. tricholomatoidea*. Scale bar = 10 µm.

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First record of *Trappea darkeri* from Turkey

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Abstract—The truffle-like basidiomycete genus *Trappea* is reported as a new record from Turkey and western Asia, based on a collection of *T. darkeri* from Niğde province. This is also the first record of the family *Trappeaceae* for the country. A brief description and photographs of the collection is provided.

Key words—*Basidiomycota,* biodiversity, *Hysterangiales,* taxonomy

Introduction

Trappea Castellano is a truffle-like fungi genus in the family *Trappeaceae* (Kirk & al. 2008). The genus was proposed by Castellano (1990), who segregated *T. darkeri* and *T. phillipsii* from *Hysterangium* Vittad. based on their smooth bacilloid basidiospores and layer of sterile locules below the peridium. *Trappea* species are characterized by subglobose to irregularly lobed fruit bodies with one or more rhizomorphs emerging from the base; a thin white peridium covering a zone of sterile chambers; an olive- brown to bright olive-green gleba with small empty chambers and a dendroid, gelatinous to cartilaginous columella; and ellipsoid to oblong basidiospores.

Index Fungorum (www.indexfungorum.org accessed 25 June 2019) lists four *Trappea* species, none of which has been reported from Turkey (Acar & al. 2019, Sesli & Denchev 2014, Solak & al. 2015, Türkekul 2017). Here we contribute to the Turkish mycobiota and report *T. darkeri* as the first representative of the genus and family in Turkey.

Materials & methods

The *Trappea* specimen was collected from Ulukışla district, Niğde province, in 2019. The basidiomata were photographed in their natural habitat, where ecological and morphological observations were taken. Dried material was examined and photographed microscopically in the fungarium using a Nikon Eclipse Ci-S trinocular compound microscope with Nikon DS-Fi2 camera and a Hitachi SU5000 scanning electron microscope. The specimen was identified by consulting the literature (Castellano 1990, Desjardin & al. 2014, Gómez-Reyes & al. 2014, Montecchi & Sarasini 2000, Ruini 1990, Zeller 1939). The collection is preserved in the fungarium of Department of Biology, Science Faculty, Karamanoğlu Mehmetbey University, Karaman, Turkey (KMU).

Taxonomy

Trappea darkeri (Zeller) Castellano, Mycotaxon 38: 3 (1990) FIG. 1

BASIDIOMA 40 mm in diam, hypogeous to semi-hypogeous, globose, surface glabrous, with basal rhizomorphs at the base, whitish, light brownish when rubbed. PERIDIUM whitish in section, not separable, membranous-leathery. GLEBA firm, bright olive-green, olive gray to light olive-brown, white near the peridium, spongy gelatinous. COLUMELLA distinct, gelatinous, translucent, typically dendroid and reaching almost throughout the sporocarp. Odor unpleasant. BASIDIA 19–25 × 5–8 µm, irregularly cylindrical to clavate, (4–)6(–8)-spored, clamp connections present. CYSTIDIA not observed. BASIDIOSPORES 4–5 × 2–3 µm, ellipsoid to cylindrical, smooth, hyaline, sessile or with a short pedicel.

SPECIMEN EXAMINED—**TURKEY, NIĞDE: Ulukışla**, Çiftehan village, 37°30′N 34°47′E, 930 m, in soil among needle-litter in pine–oak mixed forest, 23.03.2019, O.Ber 342 (KMU).

COMMENTS—*Trappea darkeri* has a hypogeous to semi-hypogeous habit with long rhizomorphs at the base and is distributed in high altitudes of 1000-3000 m (Castellano 1990, Desjardin & al. 2015, Gómez-Reyes & al. 2014). It is distinguished by a layer of sterile locules just under the peridium, and small, cylindrical spores (Gómez-Reyes & al. 2014). Morphologically it may be confused with *Hysterangium* species with rubbery-gelatinous olive-green gleba, which differ in their easily separable peridia, absence of sterile locules next to the peridium and larger, spindle-shaped spores (Desjardin & al. 2015, Siegel & al. 2019). Ecologically and morphologically similar, *Trappea pinyonensis* States can easily be distinguished from *T. darkeri* by the staining reactions of the peridium and rhizomorphs and its longer (5–6.2 × 2–2.5 µm) spores (States 1991).

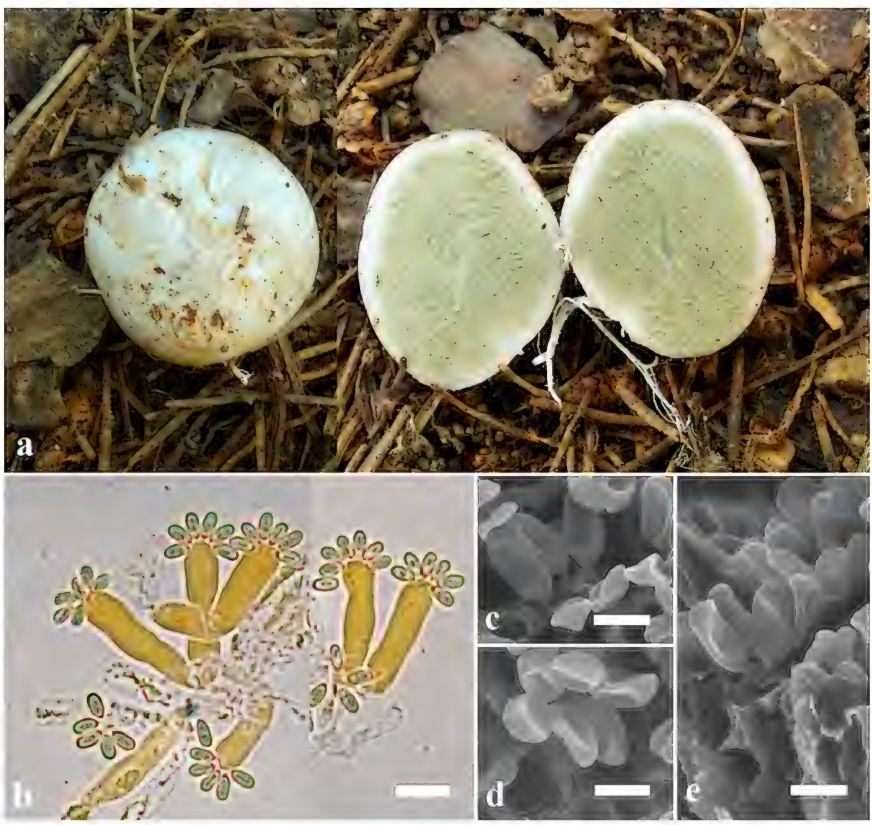

Fig. 1. *Trappea darkeri* (KMU – Ber 342). a. Basidiomata; b. Basidia and basidiospores; c–e. Basidiospores (SEM). Scale bars: b = 10 µm; c–e = 3 µm.

Although our specimen was collected at a slightly lower elevation (930 m) than cited elsewhere, its morphological characters agree with those cited in the literature (Castellano 1990, Desjardin & al. 2015, Gómez-Reyes & al. 2014, Montecchi & Sarasini 2000, Ruini 1990, Zeller 1939).

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Mesocorynespora sinensis gen. & sp. nov. from southern China

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Abstract—A new anamorphic genus and species, *Mesocorynespora sinensis* collected on decaying culms of bamboo in China, is described and illustrated. The fungus is distinguished by short, unbranched, clavate conidiophores with monotretic, conidiogenous cells that produce solitary, acrogenous, obclavate, euseptate conidia. A key to *Mesocorynespora* and morphologically similar genera is provided.

Key words—asexual fungi, hyphomycetes, taxonomy

Introduction

Lushan Mountain, located in the northern part of Jiangxi Province, China, covers approximately 302 km^2 and exhibits a unique geography with complex terrains and unusual climate. The mountain's distinctive vegetation has favored the survival and multiplication of various microbial species, yet only relatively little information has been published on saprobic hyphomycetes recorded in China (e.g., Ma & Zhang 2015, Ma 2016, Ma & al. 2016, Xu & al. 2017, Ai & al. 2019). During our continuing surveys of asexual fungi in this region, an

interesting fungus was collected that differed remarkably from all previously described hyphomycetes (Seifert & al. 2011). Thus, it is described here as a new genus and species.

Taxonomy

Mesocorynespora Jian Ma, X.G. Zhang & R.F. Castañeda, **gen. nov.**

MB 836511

Differs from *Solicorynespora* by its short, determinate, clavate conidiophores and multi-euseptate conidia; and from *Corynespora* by its euseptate conidia.

Type species: *Mesocorynespora sinensis* Jian Ma & al.

Etymology: Greek, *meso-* meaning middle- + Latin, *-corynespora*, referring to the genus *Corynespora*.

Conidiophores short, single or in groups, clavate, unbranched, brown. Conidiogenous cells monotretic, integrated, terminal, clavate, determinate. Conidial secession schizolytic. Conidia solitary, acrogenous, dry, obclavate, multi-euseptate.

Mesocorynespora sinensis Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** Figs 1, 2

MB 836512

Differs from *Solicorynespora* spp. by its short, determinate conidiophores with multi-euseptate conidia, and from *Corynespora* spp. by its euseptate conidia.

Type: China, Jiangxi Province: Lushan Mountain, on decaying culms of bamboo, 9 November 2014, J. Ma (**holotype**, HJAUP M0296).

Etymology: refers to China where the fungus was collected.

Colonies on natural substrate effuse, brown to dark brown. Mycelium superficial and immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae. Conidiophores short, single or in groups, erect, straight or flexuous, clavate, unbranched, brown, smooth, 1–3-septate, 25–40 × 3.5–5 µm. Conidiogenous cells monotretic, integrated, terminal, determinate, clavate, brown, smooth, 9–14.5 × 5–6.5 µm. Conidial secession schizolytic. Conidia solitary, dry, acrogenous, obclavate, smooth, brown to dark brown, rounded and golden yellow at the apex, truncate at the base, 20–35-euseptate, 72–171 ×13.5–16 µm, tapering to 7–10.5 µm diam. at the apex, 4–5 µm diam. at the base.

Discussion

Mesocorynespora sinensis is unique in having short, clavate, unbranched conidiophores, and solitary, acrogenous, obclavate, multi-euseptate conidia

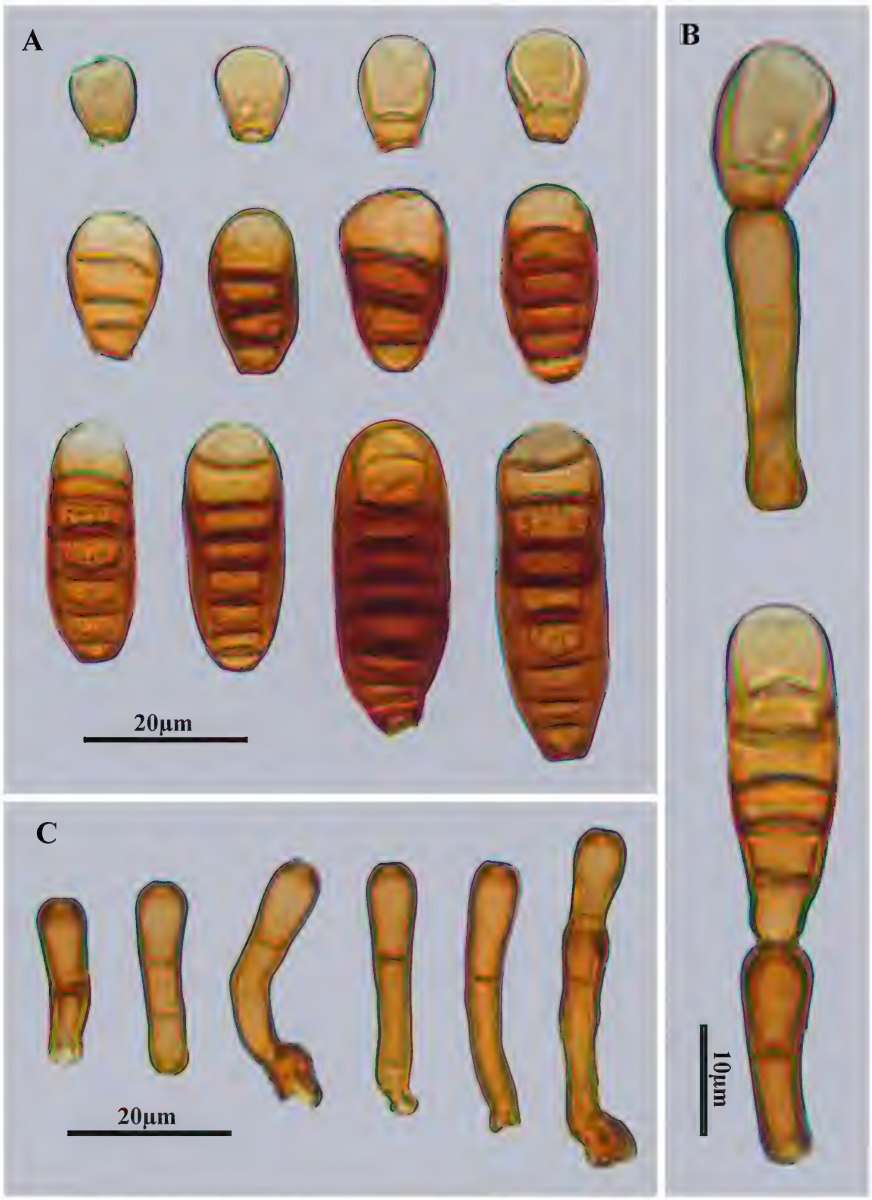

Fig. 1. *Mesocorynespora sinensis* (holotype, HJAUP M0296).
A. Developing conidia; B. Conidiophores with developing conidia;
C. Conidiophores, and conidiogenous cells.

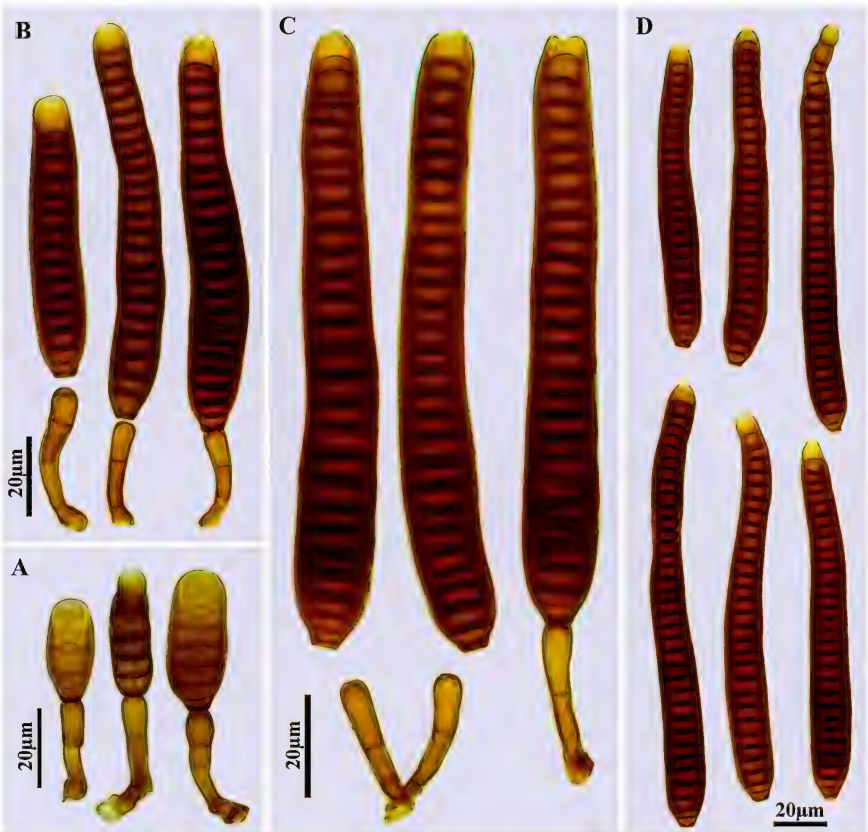

Fig. 2. *Mesocorynespora sinensis* (holotype, HJAUP M0296).
A–C. Conidiophores, conidiogenous cells, and conidia; D. Conidia.

that secede schizolytically from the monotretic, integrated, terminal, determinate conidiogenous cells. We initially thought of assigning it to the genus *Solicorynespora* R.F. Castañeda & W.B. Kendr. (Castañeda-Ruíz & Kendrick 1990), but *Solicorynespora* species have long, cylindrical to obclavate conidiophores with percurrently extending conidiogenous cells and conidia with fewer septa.

Several other genera including *Corynespora* Güssow, *Corynesporella* Munjal & H.S. Gill, *Hemicorynespora* M.B. Ellis, *Corynesporopsis* P.M. Kirk, and *Corynesporina* Subram. (Güssow 1905, Munjal & Gill 1961, Ellis 1972, Kirk 1981, Gams & al. 2009) have the same conidiogenesis as *Mesocorynespora*, but *Mesocorynespora* differs in having clavate, determinate conidiogenous cells

formed from short, clavate, unbranched conidiophores; and differs further from *Corynespora*, *Corynesporella*, and *Corynesporina* by its euseptate conidia and from *Corynesporopsis* by its solitary conidia. Conidia in *Hemicorynespora* are 0–1-septate, an obvious difference from *Mesocorynespora*.

Key to *Mesocorynespora* and morphologically similar genera

1. Conidiophores short, conidiogenous cells without percurrent extension ..... *Mesocorynespora*
1. Conidiophores long, conidiogenous cells with percurrent extension 2
2. Conidia aseptate or euseptate 3
2. Conidia distoseptate 5
3. Conidia catenate ..... *Corynesporopsis*
3. Conidia solitary 4
4. Conidia aseptate or with a single septum ..... *Hemicorynespora*
4. Conidia two- to pluriseptate ..... *Solicorynespora*
5. Conidiogenous cells formed as terminal and lateral branchlets on the conidiophores ..... *Corynesporella*
5. Conidiogenous cells integrated, terminal on the conidiophores 6
6. Conidia formed single or in acropetal chains ..... *Corynespora*
6. Conidia solitary and in basipetal chains ..... *Corynesporina*

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Corynesporopsis hainanensis sp. nov., a bambusicolous fungus from southern China

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Abstract—A new anamorphic species, *Corynesporopsis hainanensis*, is described and illustrated from dead bamboo culms collected in the tropical mountain rainforest of Jianfengling, Hainan, China. The fungus is characterized by its terminal monotretic conidiogenous cells with catenate, obclavate, brown to pale brown, smooth, (3–)5–6(–10)-euseptate conidia. A dichotomous key and a synoptic table to *Corynesporopsis* species are provided.

Key words—asexual fungi, hyphomycetes, saprobes, taxonomy, *Xylariales*

Introduction

Taking into account its large area, varied geographical conditions and advantageous natural environments, China is considered an important reservoir of the original bamboo diversity (Yang & Xue 1998). Its bamboo forest ecosystems offer favorable habitats for survival and multiplication of bambusicolous fungi. However, our knowledge of bambusicolous fungi is scant, and relatively little information about their taxonomy has been published in China (e.g. Zhou & al. 2001, Xu & al. 2006, 2007, Ma 2016). During our ongoing surveys of saprobic microfungi associated with plant debris, we

collected on dead bamboo culms an interesting hyphomycete with the typical morphological features of *Corynesporopsis* P.M. Kirk (Kirk 1981a). The fungus, significantly different from previously described taxa, is proposed here as new to science.

Materials & methods

Samples of litter were placed in paper and plastic bags, taken to the laboratory, and prepared according to Castañeda-Ruiz & al. (2016). Mounts were prepared in PVL (polyvinyl alcohol and lactic acid) and measurements were made at a magnification of ×1000. Micrographs were obtained with a Nikon Eclipse 80i microscope equipped with bright field and Nomarski interference optics. The type specimen was deposited in the Herbarium of Jiangxi Agricultural University, Nanchang, Jiangxi, China (HJAUP).

Taxonomy

Corynesporopsis hainanensis Z.H. Xu, Jian Ma, X.G. Zhang & R.F. Castañeda, **sp. nov.** Fig. 1

MB 836568

Differs from *Corynesporopsis curvularioides*, *C. iberica,* and *C. rionensis* by its (3–)5–6(–10)-euseptate obclavate conidia; from *C. iberica* by its wider conidia; and from *C. curvularioides* and *C. rionensis* by its longer and narrower conidia.

Type: China, Hainan Province, Jianfengling National Nature Reserve, on dead culms of bamboo, 18 April 2014, J. Ma (**Holotype**, HJAUP M0377).

Etymology: refers to the province where the type was collected.

Colonies on dead wood effuse, dark brown to black, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth hyphae. Conidiophores differentiated, single, erect, unbranched, straight or slightly flexuous, brown to dark brown, smooth, septate, 45–62 × 5.5–6.5 µm, thick-walled. Conidiogenous cells monotretic, integrated, terminal, determinate, cylindrical, brown, smooth, 10–13 µm long, 4.5–5.5 µm wide. Conidial secession schizolytic. Conidia acrogenous, catenate, in unbranched, acropetal chains, obclavate, straight or curved, brown to pale brown, smooth, (3–)5–6(–10)-euseptate, 17–48 µm long, 6.5–8 µm diam. in the broadest part, 2–3 µm diam. at the truncate base.

Dissussion

Corynesporopsis was established by Kirk (1981a) with *Corynespora quercicola* Borowska [≡ *Corynesporopsis quercicola* (Borowska) P.M. Kirk] as the type species, and was mainly characterized by acrogenous, catenate, euseptate conidia seceding schizolytically from monotretic, integrated,

FIG. 1. *Corynesporopsis hainanensis* (holotype, HJAUP M0377).
A, B. Conidiophores, conidiogenous cells, and conidia; C. Conidiophores; D. Conidia.

terminal, determinate or rarely percurrently extending conidiogenous cells. It is similar to *Corynespora* Güssow, *Corynesporella* Munjal & H.S. Gill, *Hemicorynespora* M.B. Ellis, *Solicorynespora* R.F. Castañeda & W.B. Kendr., and *Corynesporina* Subram. in having monotretic conidiogenous cells (Güssow 1905, Munjal & Gill 1961, Ellis 1972, Castañeda-Ruíz & Kendrick 1990, Gams & al. 2009). However, *Corynesporopsis* differs from *Hemicorynespora* and *Solicorynespora* by its catenate conidia and from *Corynespora*, *Corynesporella*,

Table 1 Comparisons of conidia and substrates of *Corynesporopsis* species. The new species is set in **bold** font.

Species	Conidia			Habitat	Type locality
	Size (μm)	Shape and Septation	Color		
C. acaciae [1]	(10–)11–13 × (4.5–)5(–5.5)	Ellipsoidal to cylindrical, broadly rounded at the ends, 1-euseptate	Uniform	Dead wood	China
C. antillana [2]	21–28(–33) × 5–8	Ellipsoidal, (3–)5(–6)-euseptate	Variable	Dead leaves	Cuba
C. biseptata [3]	18–33 × 7–9	Cylindrical, mostly 2-euseptate	Uniform	Dead wood	UK
C. curvularioides [4]	15–35 × 7–11	Obclavate to ellipsoidal, 1–5-euseptate	Uniform	Dead branches	China
C. cylindrica [5]	12.5–20.5 × 6–7.5	Cylindrical, 1(–2)-euseptate	Uniform	Dead wood	Australia
C. excoecariae [6]	15–40 × 7.5–10.5	Cylindrical, 1–3-euseptate	Uniform	Dead branches	China
C. hainanensis	**17–48 × 6.5–8**	**Obclavate, (3–)5–6(–10)-euseptate**	**Uniform**	**Dead branches**	**China**
C. iberica [7]	15–48(–59) × 3–4	Cylindrical, (2–)3–7-euseptate	Uniform	Bark	Portugal
C. inaequiseptata [8]	17–25 × 4–5.5	Obclavate, 1-euseptate	Variable	Rotten palm petiole	Peru
C. indica [9]	(14–)17–24(–27) × 8–12(–14)	Ellipsoid to broadly obovoid, 1-euseptate	Uniform	Dead twig	India
C. isabelicae [10]	(24–)27–43.5 × 4–6.4	Elongate fusiform or elongate naviculiform, 1-euseptate	Uniform	Rotten palm petiole	Cuba
C. liquidambaris [11]	10–16.5 × 3.5–5.5	Naviculiform to ellipsoid, 1-euseptate	Uniform	Dead branches	China
C. obclavata [12]	27–61 × 6–12	Obclavate, 5–9-euseptate	Uniform	Dead branches	China
C. quercicola [13]	(12–)14–18 × 6–8(–9)	Broadly ellipsoid to cylindrical, 2-euseptate	Variable	Decaying wood	Poland
C. rionensis [14]	24–36 × 8–11 (mostly 9–10)	Fusiform, broad fusiform or ellipsoidal, 3–4(–5)-euseptate	Uniform	Rotten branches	Cuba
C. uniseptata [15]	12–16 × 5–7	Ellipsoid, 1-euseptate	Uniform	Dead leaves	UK
C. variabilis [16]	11–16 × 5–8	Ellipsoid, obclavate, sub-oblong, cylindrical, sub-pyriform to oval, 1-euseptate	Variable	Submerged dead wood	Spain

Data from: [1] Kirschner 2015; [2] Castañeda-Ruiz & Kendrick 1990; [3] Morgan-Jones 1988; [4] Xia & al. 2013; [5] Sutton 1989; [6] Xia & al. 2014; [7] Castañeda-Ruiz & al. 2011; [8] Matsushima 1993; [9] Kirk 1983; [10] Holubová-Jechová 1987; [11] Ma & al. 2012; [12] Ma 2016; [13] Kirk 1981a; [14] Holubová-Jechová & Mercado Sierra 1986; [15] Kirk 1981b [16] Hernández-Restrepo & al. 2014.

and *Corynesporina* by its euseptate conidia. *Corynesporopsis* is also similar to *Heteroconium* Petr. (Petrak, 1949) in having catenate, euseptate conidia, but *Heteroconium* is separated by monoblastic conidiogenesis.

Although *Corynesporopsis* was placed in *Xylariales* (*Sordariomycetes*) based on ITS and LSU rDNA based phylogenetic studies of *C. acaciae* R. Kirschner, its family remains incertae sedis due to its tretic conidiogenesis, previously unrecorded for *Xylariales* (Kirschner 2015). Teleomorphs and phylogenetic affinities with other *Corynesporopsis* species are not yet known.

Among the known species of *Corynesporopsis*, only *C. curvularioides* J.W. Xia & X.G. Zhang (Xia & al. 2013), *C. iberica* R.F. Castañeda & al. (Castañeda-Ruíz & al. 2011), and *C. rionensis* Hol.-Jech. (Holubová-Jechová & Mercado Sierra 1986) are comparable to *C. hainanensis*. However, *C. curvularioides* differs in its obclavate to ellipsoidal, shorter and wider conidia with 1–5 eusepta; *C. iberica* differs in its cylindrical, narrower conidia with (2–)3–7 eusepta; while *C. rionensis* differs in its wider fusiform, broadly fusiform, or ellipsoidal conidia with 3–4(–5) eusepta.

With the addition of *C. hainanensis*, *Corynesporopsis* currently contains 17 species. A synopsis for comparisons of conidia and substrates of these accepted species is presented in Table 1. The conidia of these 17 *Corynesporopsis* species are mostly ellipsoidal, cylindrical, obclavate, or fusiform, but conidia may also be obovoid, naviculiform, sub-oblong, sub-pyriform, or oval. The conidia are mostly unicolored, but *C. antillana* R.F. Castañeda & W.B. Kendr., *C. inaequiseptata* Matsush., *C. quercicola*, and *C. variabilis* R.F. Castañeda & al. produce versicolored conidia. Eight species produce predominately 1-septate conidia, usually thickened and darkened at the septa—*C. acacia, C. cylindrica* B. Sutton, *C. indica* P.M. Kirk, *C. isabelicae* Hol.-Jech., *C. liquidambaris* Jian Ma & X.G. Zhang, *C. uniseptata* P.M. Kirk, *C. inaequiseptata*, and *C. variabilis*—while the other *Corynesporopsis* species produce conidia with more septa. All species were found as saprobes on bark, decaying leaves, rotten wood, or dead palm rachides in terrestrial environments, except for *C. variabilis* on submerged dead wood. Fourteen species definitely produce smooth-walled conidia; the conidial ornamentation of the other three species (*C. uniseptata*, *C. curvularioides* and *C. excoecariae* J.W. Xia & X.G. Zhang) is inexplicably omitted in their original descriptions (Kirk 1981b, Xia & al. 2013, 2014).

Keys to species of *Corynesporopsis* have been provided by Castañeda-Ruiz & al. (2011) and Hernandez-Restrepo & al. (2014), based on conidial morphology. The following expanded key includes all 17 accepted *Corynesporopsis* species.

Key to *Corynesporopsis* species

1. Conidia predominantly 1-euseptate 2
1. Conidia predominantly 2 or more septa 9
2. Conidia versicolorous 3
2. Conidia concolorous 4
3. Conidia obclavate, 17–25 × 4–5.5 µm ..... *C. inaequiseptata*
3. Conidia variable ellipsoid, obclavate, sub-oblong, cylindrical, sub-pyriform to oval, 11–16 × 5–8 µm ..... *C. variabilis*
4. Conidia (14–)17–24(–27) × 8–12(–14) µm ..... *C. indica*
4. Conidia not exceeding 7.5 µm diam. 5
5. Conidia (24–)27–43.5 × 4–6.4 µm, elongate fusiform or elongate naviculiform ..... *C. isabelicae*
5. Conidia not exceeding 20.5 µm long 6
6. Conidia strictly cylindrical or ellipsoidal 7
6. Conidia ellipsoidal to cylindrical, or naviculiform to ellipsoid 8
7. Conidia cylindrical, 12.5–20.5 × 6–7.5 µm ..... *C. cylindrica*
7. Conidia ellipsoidal, 12–16 × 5–7 µm ..... *C. uniseptata*
8. Conidia ellipsoidal to cylindrical, (10–)11–13 × (4.5–)5(–5.5) µm ..... *C. acaciae*
8. Conidia naviculiform to ellipsoid, 10–16.5 × 3.5–5.5 µm ..... *C. liquidambaris*
9. Conidia versicolorous 10
9. Conidia concolorous 11
10. Conidia ellipsoidal, 21–28(–33) × 5–8 µm, (3–)5(–6)-euseptate ..... *C. antillana*
10. Conidia broadly ellipsoid to cylindrical, (12–)14–18 × 6–8(–9) µm, 2-euseptate ..... *C. quercicola*
11. Conidia cylindrical 12
11. Conidia fusiform, ellipsoidal, obclavate, or obclavate to ellipsoidal 14
12. Conidia 15–48(–59) × 3–4 µm, (2–)3–7-euseptate ..... *C. iberica*
12. Conidia at least 7 µm wide 13
13. Conidia 18–33 × 7–9 µm, mostly 2-euseptate ..... *C. biseptata*
13. Conidia 15–40 × 7.5–10.5 µm, 1–3-euseptate ..... *C. excoecariae*
14. Conidia strictly obclavate 15
14. Conidia fusiform, ellipsoidal, or obclavate to ellipsoidal 16
15. Conidia 17–48 × 6.5–8 µm, (3–)5–6 (–10)-euseptate ..... *C. hainanensis*
15. Conidia 27–61 × 6–12 µm, 5–9-euseptate ..... *C. obclavata*
16. Conidia obclavate to ellipsoidal, 15–35 × 7–11 µm, 1–5-euseptate ..... *C. curvularioides*
16. Conidia fusiform or ellipsoidal, 24–36 × 8–11 (mostly 9–10) µm, 3–4(–5)-euseptate ..... *C. rionensis*

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Hymenochaete longisterigmata sp. nov. from India

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Abstract—A new hymenochaetoid species, *Hymenochaete longisterigmata,* is described from Himachal Pradesh, India.

Keywords—*Basidiomycota*, *Hymenochaetales*, *Hymenochaetaceae,* Himalaya

Introduction

During the 2014 fungal forays conducted in the Narkanda area of district Shimla, Himachal Pradesh, India, Navpreet & Dhingra collected a fungal specimen on the branch of a living tree of *Picea smithiana*. This fungus was again collected (2016) in the Jalori Pass area of district Kullu, Himachal Pradesh, India, by Navpreet & Avneet on the branch of a living *Quercus semecarpifolia*. Based on the comparison of morphological characters (Cunningham 1963, Sharma 1995 & 2012, Natarajan & Kolandavelu 1998, Bernicchia & Gorjón 2010, Dai 2010, Kaur & al. 2015, Sharma & Mishra, 2015, Mycobank 2019), we referred the specimens to *Hymenochaete* Lév. (*Hymenochaetaceae*) and close to *H. corrugata* (Fr.) Lév. based on its resupinate, adnate basidiomata, heavily cracked hymenial surface, and presence of abundant setae with encrusted apices. We now propose the fungus as a new species *H. longisterigmata,* named in reference to its exceptionally long sterigmata.

Materials & methods

The present study is based on fungal samples collected during the field trips conducted in Narkanda (District Shimla) and Jalori Pass (District Kullu) forest areas of Himachal Pradesh during September 2014 and 2016. Colours terms follow Kornerup

& Wanscher (1978). After removing any extraneous matter, we dried the specimens in the sun or with a portable electric drier. The dried specimens were deposited at the internationally recognized herbarium of Department of Botany, Punjabi University, Patiala, India (PUN). The arrangement, size and shape of hyphae, sterile structures, basidia, and basidiospores were studied by making crush mounts and free hand sections in water, KOH (3%/5%/10%), 1% Congo red, 1% phloxine, cotton blue (15 in Lactophenol), and Melzer's reagent. We examined the material microscopically at 100×, 400× and 1000× magnifications using a compound microscope and drew structural line diagrams on white paper sheets with the aid of a camera lucida. Photomicrographs of important structures were taken using a Nikon 80i Eclipse microscope.

Taxonomy

Hymenochaete longisterigmata Nav. Kaur, Avn.P. Singh & Dhingra, **sp. nov.** PLATE 1

MB 832678

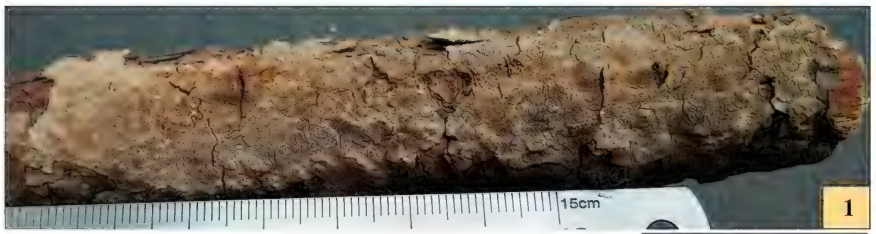
Differs from *Hymenochaete corrugata* by its thick–walled hyphidia, its larger basidia with long sterigmata, and its broadly ellipsoid basidiospores.

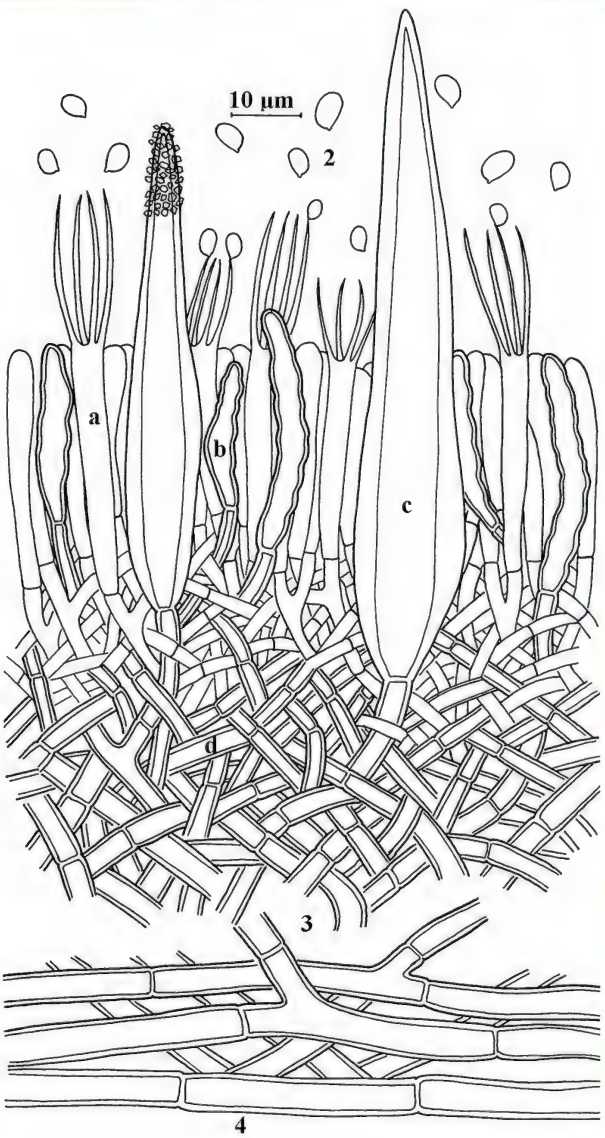
TYPE: India, Himachal Pradesh, Shimla, Narkanda, about 3 Km from Narkanda towards Baghi, on branch of living tree of *Picea smithiana* (Wall.) Boiss., 4 September 2014, Navpreet & Dhingra 9689 (**Holotype**, PUN).

ETYMOLOGY: The specific epithet refers to the long sterigmata.

Basidiomata annual, resupinate, ≤2 mm thick in section, adnate, soft and corky when collected, hard and corky after drying; hymenial surface smooth to somewhat aculeate under lens, cracked, greyish orange to greyish brown when collected, no significant change after drying; margins thinning, indeterminate, paler concolorous, sterile portion ≤0.5 mm. Hyphal system monomitic. Subicular hyphae brown, heavily encrusted, up to 6.3 µm wide; subhymenial hyphae subhyaline to yellowish brown, up to 3.4 µm diam. Setae subulate, apices straight with crystalline encrustation, dark brown, abundant, thick–walled, 68–92 × 11.4–20 µm; arising from the subhymenium, extending through hymenium, projecting ≤52 µm above the hymenium. Hyphidia subfusiform with moniliform apices, yellowish brown, abundant, thick–walled, 20–34 × 4.2–5.2 µm; arising from the top of subhymenium and base of hymenium. Basidia narrowly clavate to subcylindrical, subhyaline, thin–walled, tetrasterigmate, 22–30 × 2.8–4.6 µm; sterigmata ≤17.2 µm long.

PLATE 1. *Hymenochaete longisterigmata* (holotype, PUN – Navpreet & Dhingra 9689). 1. Basidiocarp showing hymenial surface; 2. Basidiospores; 3. Vertical section through basidiocarp showing: a. basidia; b. hyphidia; c. setae; d. subhymenial hyphae; 4. Subicular hyphae; 5. Basidium; 6. Setae.


15cm
1


10 µm
2
a
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3
4

20 µm
5

20 µm
6

Basidiospores broadly ellipsoid, subhyaline, smooth, thin–walled, 4–5.1 × 2.8–3.4 µm, inamyloid, acyanophilous.

Additional specimen examined: **INDIA**, **Himachal Pradesh**, **Kullu**, about 3 km from Jalori Pass towards Shoja, on branch of living tree of *Quercus semecarpifolia* Sm., 3 September 2016, Navpreet & Avneet 9688 (PUN).

Discussion

Hymenochaete (*Hymenochaetaceae*) is a widespread genus with c. 350 published species names and c. 150 currently accepted species (Kirk & al. 2008, Index Fungorum 2019, MycoBank 2019). The earliest record of this genus from India was by Montagne (1842), who described *Stereum rheicolor* [≡ *H. rheicolor*] from Gudalur, Nilgiri Hills, Tamil Nadu. Until our report, 40 *Hymenochaete* taxa were reported from India.

Wagner & Fischer (2002) inferred phylogenetic relationships from DNA sequence data for several *Hymenochaete* species. Their phylogeny supporting traditional *Hymenochaete* species in two genera, they described the new genus *Pseudochaete* T. Wagner & M. Fisch. The two genera are not easily distinguished by morphology alone. The new species described here has not been sequenced and is therefore referred to *Hymenochaete* s.l.

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Entoloma conferendum, Hygrocybe coccineocrenata, and *Hypholoma ericaeum* new to Montenegro

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Abstract—As a result of the first research on macrofungi on peat moss habitats in Montenegro, three species new to the Montenegrin mycobiota are presented and described: *Entoloma conferendum*, *Hygrocybe coccineocrenata*, and *Hypholoma ericaeum*. The descriptions are accompanied by photographs of the basidiomata on site and some microscopic characters. Additionally, the second Montenegrin record of *Bovista paludosa* is presented and described, for the first time with locality and habitat data. Species identifications were checked by comparing ITS rDNA sequences from samples with the sequences in GenBank.

Key words—*Agaricales, Basidiomycota*, biodiversity, taxonomy

Introduction

Sphagnum (peat moss) is a genus with several hundred species that live in wet habitats and can store water in their cells. The most important habitats are peat bogs, where due to constant water availability and oxygen reduction, peat sediments form by the accumulation of the partially decomposed plant remnants. Peat bogs are characterised by acidic soils, low nutrient content,

and dominance of peat mosses. These habitats, which form in humid and cold climates, are widely distributed in northern parts of Asia, Europe, and North America. However, despite a still favourable climate, natural peatlands are vanishing from northern Europe due to human activities such as peat extraction, drainage, and land development. In the western Balkans, primarily due to unfavourable climate conditions, peat bogs are exceptionally rare and relict habitats, occupying only small highly isolated areas. They are particularly sensitive to climate changes, which can cause them to dry out and become overgrown by other vegetation types. Therefore, peat bogs are one of the most endangered ecosystems in Montenegro. Some macrofungal species live exclusively in this type of habitat, but a certain number of fungi with a wider habitat tolerance can also be found in peat bogs. Peat mosses can also live in wet, acidic habitats without forming peat, mostly in coniferous forests.

Due to the small number of researchers, Montenegrin fungi, including agarics, are rather poorly explored. Kasom (2013) lists 839 macro-basidiomycete species for Montenegro, 444 of which belong to the *Agaricales*. Bubák (1915), who conducted the first mycological research on Montenegrin peat bogs in 1904, reported several microfungi from the Barno jezero peat bog (Mt. Durmitor). However, until our research, no macrofungi had been reported in peat bogs of Montenegro. During the summer and autumn of 2017 and 2018 we surveyed macrofungi of mountain peat bogs and peat moss carpets on Mt. Sinjajevina, Mt. Durmitor, and Mt. Hajla. We describe three *Agaricales* species new to Montenegrin mycobiota, as well as one previously reported rare species with the first data on its locality and habitat in Montenegro.

Materials & methods

Collected basidiomata were photographed on the site, described, preserved by drying and deposited in the mycological collections of Natural History Museum of Montenegro, Podgorica (NHMP) and/or Croatian National Fungarium in Zagreb (CNF).

The morphological descriptions are entirely based on the collected material. Microscopic features are described from dried material mounted in 2.5% potassium hydroxide (KOH) and observed with a light microscope under magnification up to 1500×. Basidiospore measurements were made by Motic Images Plus 2.0 software on 30 randomly chosen mature basidiospores from calibrated digital images. Arithmetic means of basidiospore length and width are shown in italic font in the centre of the measurements. The length/width ratio of all measured basidiospores is given as the "Q" value (min. – av. – max.).

Genomic DNA was extracted from dried specimens using DNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions. PCR primers ITS1F and

ITS4 (Gardes & Bruns 1993) were used for ITS region amplification and sequencing purposes. The ITS rDNA regions were amplified in a thermocycler (Eppendorf) with a final volume of 25 µL containing approximately 50 ng DNA template, 0.2 µM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1× buffer, and 1 unit of Taq polymerase (Invitrogen). The cycling protocol was: 94 °C for 5 min, 30 cycles (94 °C for 45, 60 °C for 45 s, 72 °C for 45 s), followed by a final extension of 72 °C for 10 min. The PCR products were resolved on a 1% agarose gel stained with ethidium bromide and viewed under ultraviolet light. PCR products were purified and sequenced by Macrogen Inc. (The Netherlands). Sequences were aligned in both directions and edited using Sequencher v.4.1.4 and submitted to GenBank.

Taxonomy

Hygrocybe coccineocrenata (P.D. Orton) M.M. Moser, Röhrlinge & Blätterpilze, 3. Aufl.: 68 (1967). FIG. 1 A, B

PILEUS 6–23 mm broad, convex at first, later applanate to concave with a depressed centre and mostly inflexed and somewhat crenate margin, scarlet red, orange red or reddish orange, densely covered with small squamules or tufts which are reddish brown to grey towards the centre and concolorous towards the margin, not translucently striate, dry. LAMELLAE decurrent, distant, broad, white when young, wax yellow at maturity. STIPE 25–70 × 2–5 mm, cylindrical or tapering downwards, scarlet red to orange red, orange near the base, whitish in the base, glabrous, hollow, dry. CONTEXT thin, pale orange red. SMELL and TASTE indistinct. SPORE-PRINT white. BASIDIOSPORES 9.2–*11*–12.1 × 5.3–*6.1*–6.8 µm, Q = 1.51–*1.81*–2.13, ellipsoid to oblong, often slightly phaseoliform in side view, sometimes with slight median constriction, thin-walled, hyaline. BASIDIA 4-spored, narrowly clavate. CYSTIDIA absent. PILEIPELLIS at centre and in squamules a trichoderm composed of ascending hyphae constricted at septa, with brown intracellular pigment. CLAMP CONNECTIONS present.

SPECIMEN EXAMINED—**MONTENEGRO, KOLAŠIN MUNICIPALITY**, Mt. Sinjajevina, Semolj saddle, 42.9092°N 19.2731°E, 1580 m a.s.l., in peat bog, among dense *Sphagnum subsecundum* Nees peat moss with *Carex lasiocarpa* Ehrh. sedge, 18 July 2017, leg. S. Dragićević (NHMP 368/6986; GenBank MN082022).

ECOLOGY & DISTRIBUTION—This waxcap occurs mostly in peat bogs among or near peat moss, but also in wet meadows. Widespread (but generally rare) in Europe, known also from North America and Asia (Tkalčec & al. 2008, Boertmann 2010). In the surrounding countries, *H. coccineocrenata* is recorded in Croatia and Serbia, and it is a strictly protected species in both countries. New to Montenegro.

Comments—*Hygrocybe coccineocrenata* is characterised by decurrent lamellae, predominantly red pileus and stipe (at least in young basidiomata), darker small squamules on the pileal surface, and by living in open, wet habitats (mostly peat bogs). It has often been confused with *H. turunda* (Fr.) P. Karst., which also has darker squamules on pileus, but which differs in its orange to orange yellow pileus and stipe ground colours and growth in rather dry grasslands and heathlands (Boertmann 2010). Our material fits well with the modern descriptions (e.g., Boertmann 2010, Ludwig 2012) of *H. coccineocrenata*. The GenBank BLAST analysis shows that our ITS sequence is identical with one unpublished sequence (FM208899, as "*H. turunda;*" Query Cover 87%), and very close to another sequence from the same authors (FM208865, Identity > 99%, Query Cover 87%; deposited as "*H. cantharellus*," but quite different from the other *H. cantharellus* (Schwein.) Murrill sequences in the database). We assume that both sequences were derived from misidentified samples. Our Montenegrin sequence is the only GenBank entry deposited as *H. coccineocrenata*.

Hypholoma ericaeum (Pers.) Kühner,
Bull. Trimest. Soc. Mycol. Fr. 52: 23 (1936) Fig. 1 c–g

Pileus 17–60 mm broad, paraboloid to hemispherical at first, later convex to plano-convex, often subumbonate or with central papilla, hygrophanous, not translucently striate, brown to dark reddish brown when moist, orange brown to ochre brown on drying, often paler near margin in young basidiomata, surface dry to greasy, often with fine, whitish, fibrillose veil remnants near margin in young basidiomata. Lamellae narrowly adnate, subventricose to ventricose, moderately crowded, broad, pale brownish grey when young, then mottled grey brown, finally mottled dark purplish brown, edge whitish. Stipe 35–78 × 2–7 mm, subcylindrical, often with broadened base (up to 10 mm), solid to fistulose, pale to medium brown, whitish at apex in young basidiomata, surface dry, pruinose at apex, fibrillose-furfuraceous below, mostly with white basal tomentum. Context brown in the narrow zone beneath the pileus surface and above the lamellae, pale brown in the inner part of the pileus, whitish to dark red brown in the stipe. Smell fungoid. Taste slightly bitter. Spore-print violaceous brown. Basidiospores 12.7–*13.7*–15 × 7.5–*8.2*–8.9 µm, Q = 1.56–*1.67*–1.83, ellipsoid, moderately thick-walled to thick-walled, smooth, with distinct and central germ-pore, yellow brown in KOH, light red brown in H_2O. Basidia 4-spored, narrowly clavate or

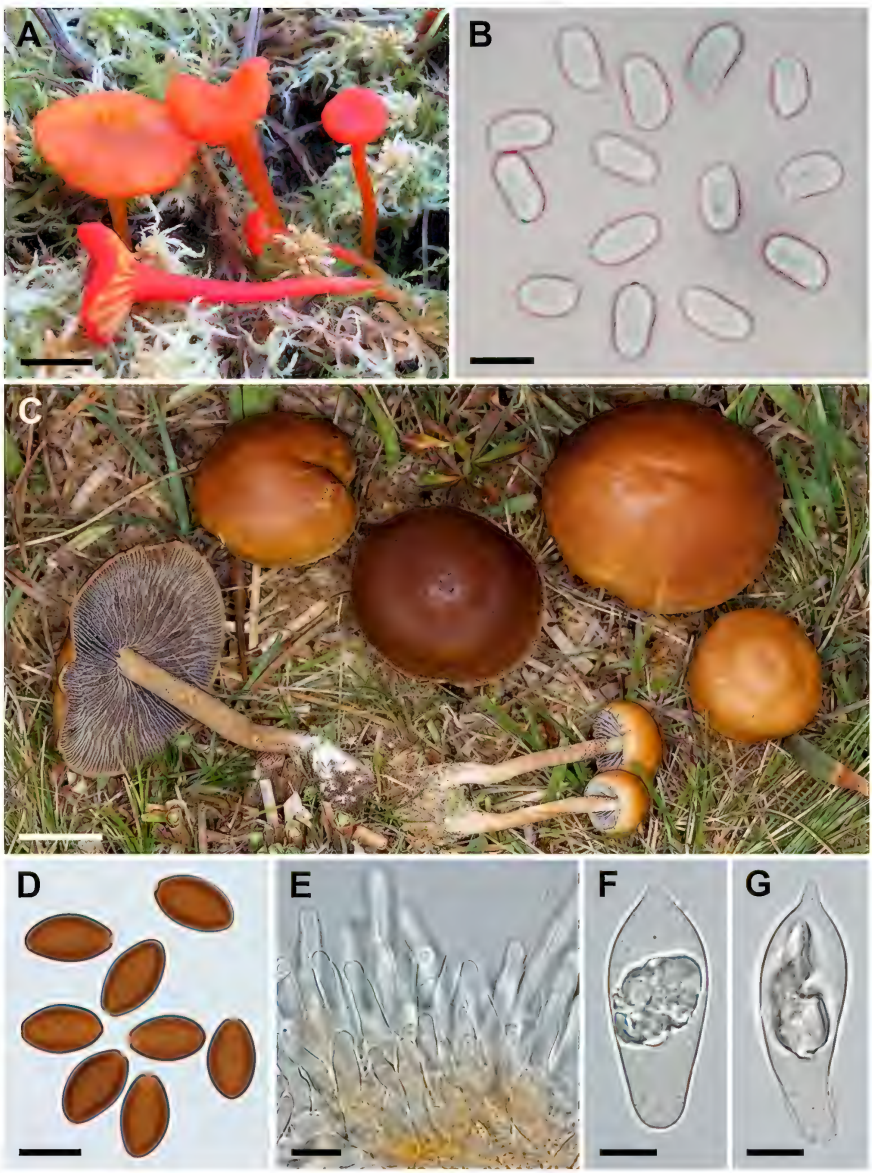

Fig. 1. ***Hygrocybe coccineocrenata*** (NHMP 368/6986). A. basidiomata; B. basidiospores. ***Hypholoma ericaeum*** (NHMP 614/9034). C. basidiomata; D. basidiospores; E. cheilocystidia; F, G. pleurocystidia. Scale bars: A = 10 mm; C = 20 mm; B, D–G = 10 μm.

with median constriction. Cheilocystidia (leptocystidia type) densely packed, forming a sterile lamellar edge, 20–45 × 5–12 µm, lageniform, narrowly utriform or subcylindrical (often with somewhat broadened apex), sometimes forked, thin-walled, hyaline. Pleurocystidia (chrysocystidia type) abundant, 30–56 × 11–18 µm, clavate, mostly with mucronate apex, thin- to moderately thick-walled, hyaline with yellowish amorphous body in alkali solution. Pileipellis a cutis composed of thin-walled, hyaline, repent hyphae. Clamp connections present.

Specimens examined—**MONTENEGRO, Kolašin Municipality**, Mt. Sinjajevina, Semolj saddle, 42.9074°N 19.2758°E, 1572 m a.s.l., among *Sphagnum* moss and grass on the edge of the peat bog, 15 September 2018, leg. Z. Tkalčec & al. (CNF 1/7623, NHMP 614/9034; GenBank MN082025); (CNF 1/7628).

Ecology & distribution—The species is saprotrophic and occurs on peaty acid soil (mostly among mosses) and in wet poor grasslands and moist heaths (Watling & Gregory 1987, Noordeloos 2011, Vesterholt & Rald 2012). It is widespread all over the boreal and temperate regions of Europe and North America (Noordeloos 2011), but usually (rather) rare. In the surrounding countries, *H. ericaeum* is recorded only in Croatia (Tkalčec & Mešić 2003). New to Montenegro.

Comments—*Hypholoma ericaeum* is characterised by long basidiospores (>13 µm) and a hygrophanous pileus that is neither translucently striate nor viscid. Only three other European *Hypholoma* species have similarly long basidiospores (*H. eximium* (C. Laest.) Rald, *H. myosotis* (Fr.) M. Lange, and *H. udum* (Pers.) Quél.), but their spore lengths reach at least 17 µm and their pilei are viscid or subviscid. Our collections correspond relatively well with recent descriptions of *H. ericaeum* (Watling & Gregory 1987, Ludwig 2001, Noordeloos 2011, Vesterholt & Rald 2012). However, the maximum stipe width of 7 mm exceeds the 4.5 mm maximum reported by Watling & Gregory (1987), the maximum cheilocystidia width of 12 µm exceeds the 7.5 µm maximum reported by Noordeloos (2011), and the maximum pleurocystidia size of 56 × 18 µm exceeds the 45 × 15 µm maximum reported by Noordeloos (2011). Moreover, Watling & Gregory (1987) described distinctly narrower cheilocystidia (30–40 × 4–5.5 µm) and pleurocystidia (30–40 × 8–10 µm). Noordeloos (2011) described somewhat flattened basidiospores, but the spores in our collections and in the descriptions of other cited authors are not flattened. According to the BLAST analysis, our ITS sequence does not match any sequence in GenBank database.

The closest sequence (96.91% identity) is derived from *H. dispersum* Quél. (HQ604746). The only GenBank ITS sequence annotated as *H. ericaeum* (MH856009) is genetically much more distant (84% identity). It is possible that MH856009 was sequenced from a misidentified sample, but it is also possible that two morphologically close species are not yet recognized as different; further research is required.

Entoloma conferendum (Britzelm.) Noordel.,
Persoonia 10: 446 (1980) FIG. 2 A, B

= *Entoloma nothofagi* G. Stev., Kew. Bull. 16: 234 (1962)

= *Entoloma staurosporum* (Bres.) E. Horak, Sydowia 28: 222 (1976)

PILEUS 10–30 mm broad, paraboloid at first, expanding to plano-conical or plano-convex with subumbonate centre, hygrophanous, brown with black centre when young, later greyish brown at centre and gradually fading to light brown towards the margin when moist, pale brownish when dry, translucently striate up to 2/3 of the radius when moist, surface dry, glabrous. LAMELLAE free, ventricose, broad, moderately crowded, white when young, pink at maturity. STIPE 35–85 × 2–4 mm, cylindrical with slightly to distinctly broadened base, densely silvery striate on pale to light grey brown background, dry. CONTEXT thin and fragile, light grey brown. SMELL and TASTE farinaceous. SPORE-PRINT dirty pink. BASIDIOSPORES 9.3–*10.8*–11.8 × 8.1–*9.4*–10.4 µm, Q = 0.95–*1.15*–1.31, cruciform-stellate, thin-walled, hyaline to pinkish. BASIDIA 4-spored, narrowly clavate. CYSTIDIA absent. PILEIPELLIS a cutis with transition to a trichoderm at centre, with intracellular brown pigment. CLAMP CONNECTIONS absent.

SPECIMEN EXAMINED—**MONTENEGRO, ROŽAJE MUNICIPALITY**, Mt. Hajla, right bank of the Ibar river, 42.7981°N 20.1083°E, 1170 m a.s.l., on dense carpet of peat moss *Sphagnum quinquefarium* (Lindb.) Warnst., on very steep (almost vertical) siliceous rocks, surrounded by mixed forest of spruce (*Picea abies*), fir (*Abies alba*) and beech (*Fagus sylvatica*), 7 September 2017, leg. A. Alegro (NHMP 426/7230; GenBank MN082023).

ECOLOGY & DISTRIBUTION—This species occurs in various habitats from the lowlands to arcto-alpine zone, including grasslands, marshes, peat bogs, damp places in deciduous and coniferous forests. It is a very common saprotroph with a wide distribution, including most temperate zones on both hemispheres (Noordeloos 1992). In the surrounding countries, *E. conferendum* is recorded only in Croatia (Mešić & Tkalčec 2003). New to Montenegro.

COMMENTS—*Entoloma conferendum* is characterised by a brown translucently striate pileus, cruciform-stellate basidiospores (unique among

European *Entoloma* species), and absence of cystidia and clamp connections. Noordeloos & Gates (2012) place it in *E.* sect. *Staurospora* in *E.* subg. *Nolanea* with species having predominantly cuboid basidiospores. *Entoloma staurosporum* is currently accepted as a synonym of *E. conferendum*. Horak (1976, 1980) synonymised *E. nothofagi,* described from New Zealand, with *E. staurosporum*, but subsequently reverted to his original opinion (Horak 1971, 1973), treating *E. nothofagi* as a separate species (Horak 2008). Noordeloos & Gates (2012), who later revised the type of *E. nothofagi*, found only minimal morphological differences from *E. conferendum*, and therefore regarded the two species as probably conspecific. This conclusion is supported by our GenBank BLAST analysis showing the RPB2 sequence of Slovakian collection of *E. conferendum* (KC710191) as almost identical with the RPB2 sequence of *E. nothofagi* from New Zealand (MH190134), with only a single base pair difference between them. There is no ITS sequence available for *E. nothofagi*. Another similar species is *E. brevispermum* G.M. Gates & Noordel. from Australia, which differs by shorter (≤9 µm long) basidiospores. Our material fits well with the modern descriptions of the species (Noordeloos 1992, Ludwig 2007, Noordeloos & Gates 2012), and our ITS sequence matched well (identity > 99%) with most *E. conferendum* sequences in GenBank.

Bovista paludosa Lév., Ann. Sci. Nat., Bot., Sér. 3, 5: 163 (1846) Fig. 2 c, d

Basidiomata pyriform, 23–48 mm high and 16–24 mm broad, sometimes with white rhizoids. Exoperidium when young white and smooth or with small, very thin, adherent, greyish, polygonal patches, later breaking up into irregular, rather large plates. Endoperidium smooth, white at first, becoming brown to olivaceous. Gleba white when young, then yellowish brown, becoming olive brown at maturity. Subgleba well developed, ≤15 mm high, compact, white when young, olive to grey brown at maturity. Spore-print olive brown. Basidiospores 4.6–*5.1*–5.6 × 4.2–*4.7*–5.4 µm, Q = 1–*1.07*–1.19, globose to subglobose (rarely broadly ellipsoid), almost smooth to asperulate, moderately thick-walled, pale yellow brown in KOH, with sterigmal remnant ≤15 µm long. Capillitium Bovista-type, non-poroid, thick-walled, rarely septate.

Specimen examined—**MONTENEGRO, Žabljak Municipality, Mt. Durmitor,** Barno jezero lake, 43.1582° N, 19.0932° E, 1495 m a.s.l., among *Aulacomnium palustre* (Hedw.) Schwägr., *Bryum pseudotriquetrum* (Hedw.) P. Gaertn. & al., and *Campylium stellatum* (Hedw.) C.E.O. Jensen mosses on the outer zone of the peat bog (less acidic), 9 July 2017, leg. I. Ćetković (NHMP 425/7229; GenBank MN082024).

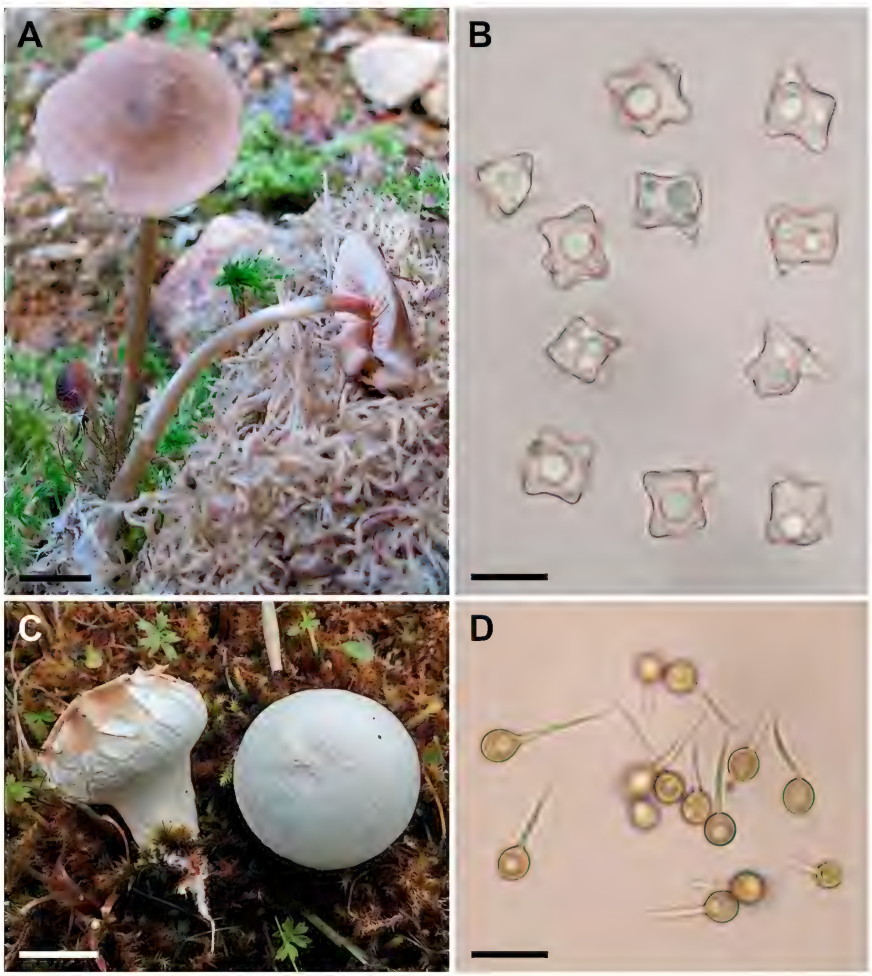

Fig. 2. ***Entoloma conferendum*** (NHMP 426/7230). A. basidiomata; B. basidiospores. ***Bovista paludosa*** (NHMP 425/7229). C. basidiomata; D. basidiospores. Scale bars: A, C = 10 mm; B, D = 10 µm.

Ecology & distribution—This puffball lives as a saprotroph in a rather wide range of open wet mossy habitats, most often in alkaline to neutral fens, bogs, mires, wet meadows, and heaths, but avoiding oligotrophic and strongly acidic mires (Kałucka 2019). The species is rather widespread but rare in Europe (Red Listed in most countries where reported), known also from Asia (China, Russia, India) and North America (Canada) (Jeppson 2018). The only earlier

Montenegrin record of *B. paludosa* from 1904 (Bubák 1915) was published without locality and habitat data. In the surrounding countries, it is recorded only in Serbia (Kałucka 2019).

Comments—*Bovista paludosa* is characterised by its occurrence on wet mossy habitats and by the presence of a well-developed subgleba, long sterigmal remnants on basidiospores, and a Bovista-type capillitium lacking pores. The most similar species, *B. cretacea* T.C.E. Fr., differs in a very poorly developed (≤2 mm high) subgleba and regularly septate capillitium. Our material agrees with the modern descriptions (Pegler & al. 1995, Sarasini 2005, Jeppson 2018). According to the BLAST analysis, our ITS sequence matched well with all three sequences of *B. paludosa* in GenBank database (Identity > 99%).

Acknowledgements

We are very grateful to Dr. André Fraiture (Botanic Garden Meise, Belgium) and Dr. Vladimír Antonín (Moravian Museum, Brno, Czech Republic) for their critical review of the manuscript. The research received financial support from the Montenegrin Ministry of Science and Croatian Ministry of Science and Education through the project "Inventory of biodiversity and conservation status of the representative peat bogs on the area of Montenegro and Croatia." Also, this work has been partially supported by Croatian Science Foundation under the project ForFungiDNA (IP-2018-01-1736).

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First Pakistani report of *Erysiphe betae* on the invasive weed *Chenopodium ambrosioides*

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Abstract—During September–October 2017, powdery mildew symptoms were observed on both surfaces of leaves of *Chenopodium ambrosioides* in Abbottabad, Malakand, and Upper Dir districts of Pakistan. The causal agent was identified as *Erysiphe betae*, based on its asexual morphology and chasmothecia, and its identity was confirmed by molecular data. This is the first Pakistani report of *Erysiphe betae* on this host.

Key words—*Amaranthaceae*, *Erysiphaceae*, mexican tea, new record

Introduction

Fungi belonging to *Erysiphales* are widely distributed all over the world and cause serious economic damage on wild and cultivated plants. This order comprises the single family *Erysiphaceae* (powdery mildews) represented by 17 genera and c. 820 species (Braun & Cook 2012). Powdery mildews infect 9839 angiosperm species in 1617 genera, 196 families, and 44 orders (Amano 1992, Takamatsu 2004), including numerous economically important plants. Various powdery mildews may cause serious economic damage on wild and cultivated plants in Pakistan (Burni & al. 2010, Mukhtar & al. 2012,

2013). Nineteen of the 56 powdery mildew species reported from Pakistan represent the most common genus, *Erysiphe,* which infects 52 hosts in 19 plant families (Ahmad & al. 1997, Burni & al. 2010, Mukhtar & al. 2013). This includes *Erysiphe betae* on *Chenopodium botrys* L. and *Spinacia oleracea* L. (*Amaranthaceae;* Amano 1986).

In 2017, a powdery mildew appeared on the leaves of *Chenopodium ambrosioides* in the districts Abbottabad, Malakand, and Upper Dir, Pakistan. The infected leaf surfaces were fully covered with white powdery mycelial masses, asexual conidia, and sexual chasmothecia indicating progressed stages of infection and severity.

Materials & methods

Sample collection

During phytopathological surveys during September–October 2017, we observed powdery mildew infections on leaves and stems of *Chenopodium ambrosioides* in the districts Abbottabad, Malakand, and Upper Dir, Khyber Pakhtunkhwa, Pakistan. The infected plants were shade dried on blotting paper and protected in brown envelopes for future use. The specimens are deposited at the herbarium of the University of Peshawar, Khyber Pakhtunkhwa, Pakistan (PUP) and Department of Botany, University of the Punjab, Lahore, Punjab, Pakistan (LAH).

Morphology

We first examined infected leaves under a Labomed CSM2 stereomicroscope, and then prepared using lactic acid. We examined the hyphae on the host; hyphal appressoria; conidial, conidiophore, and chasmothecial shapes and sizes; and ascospores and asci under a Nikon YS 100 microscope. Measurements (20 repetitions per structure) were recorded using a Lomo Filar Eyepiece Micrometer AM9-2-15x on a Zeiss microscope. Micrographs were made using a HDCE-5X digital camera.

DNA extraction, PCR amplification, phylogenetic analysis

Dried powdery mildew infections were scraped off from fresh fungal specimens with sterile razor blades, ground in liquid nitrogen, and stored in Eppendorf tubes at –18 °C. DNA was extracted using GeneJET Plant Genomic DNA Purification Mini Kit #K0791 according to the manufacturer's instructions. The Internal Transcribed Spacer (ITS) region was amplified using PMITS1/PMITS2 primers (Cunnington & al. 2003) and then commercially sequenced by Tsingke in China. Raw sequence data were edited using BioEdit (Hall 1999). The ITS sequences were BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov) and aligned using Muscle E multiple alignment tool within MEGA v. 7.0 (Kumar & al. 2016). Twenty ITS sequences were analyzed using the maximum likelihood (ML) method based on the Tamura 3-parameter model (Tamura 1992). The initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise

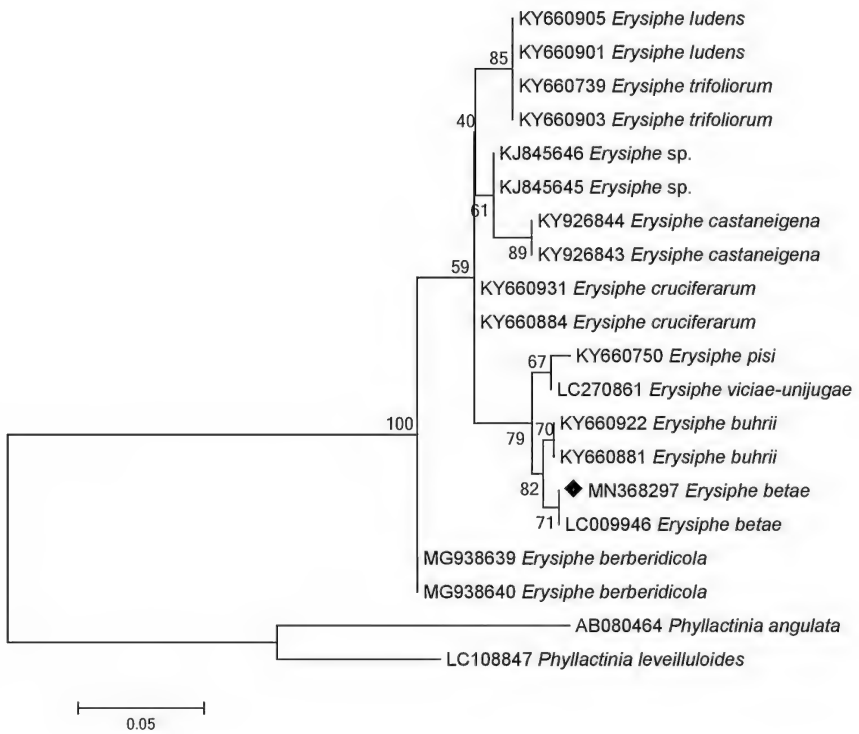

FIG. 1. Phylogenetic analysis of the ITS region for 18 *Erysiphe* sequences, with *Phyllactinia angulata* and *P. leveilluloides* as outgroup. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree with the highest log likelihood (–470.6292) is shown, and the amplified sequence from Pakistan is denoted by ♦.

distances estimated using the Maximum Composite Likelihood (MCL) approach. Evolutionary analyses were conducted in MEGA6 (Tamura & al. 2013). in the phylogenetic analyses. After elimination of gaps and missing data, the final dataset comprised 134 positions, and *Phyllactinia angulata* (E.S. Salmon) S. Blumer and *P. leveilluloides* Moreno-Rico & U. Braun served as outgroup. All sequences were aligned and trimmed at conserved sites from both 5′ and 3′ ends. The phylogenetic tree with the highest log likelihood (–470.6292) is shown in FIG. 1; the percentage of trees in which the associated taxa clustered together is shown next to the branches, and the tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Fig. 2. *Erysiphe betae* (PUP Bot.01). A. Infected plant, *Chenopodium ambrosioides*; B. Fungal mycelium under stereomicroscope; C. Conidia; D. Conidiophore; E. Chasmothecium; F. Germinating Conidium; G. Asci with ascospores; H. Ascospores; I. Chasmothecial appendage. Scale bars: A = 1 cm, B = 2 mm, C, D, F, G = 15 µm, E, H, I = 10 µm.

Taxonomy

Erysiphe betae (Vaňha) Weltzien, Phytopathol. Z. 47: 127 (1963) FIG. 2

Mycelium amphigenous, forming dense, thick, white patches; hyphae hyaline, smooth, thin-walled; hyphal appressoria nipple-shaped to somewhat lobed, 2–5 µm diam. Conidiophores arising from the top of mother cells, 43–87 × 3.5–7.5 µm; foot cells cylindrical, straight or sometime curved, 22–61 × 4.5–8 µm, constricted at the basal septum, followed by 1–2 shorter cells; conidia formed singly, ellipsoid-ovoid to cylindrical-doliiform, 14.5–51 × 7–12 µm; germ tubes arising from one end, tips slightly swollen; Chasmothecia scattered to gregarious, globose to subglobose, light to dark brown, 48.5–104 µm diam.; peridium cells small, 12–27 µm diam., irregularly polygonal; appendages numerous, mycelioid, irregularly branched, septate, thin-walled, at first hyaline but pigmented when chasmothecia mature, 29–119 × 2.5–4.5 µm; asci 3–5, ellipsoid-obovoid, 28–49.5 × 19.5–29.5 µm, stalked, 4–5-spored; ascospores globose-ellipsoid to ovoid, 8.5–18.5 × 5.5–10.5 µm, colorless.

SPECIMENS EXAMINED: **PAKISTAN, KHYBER PAKHTUNKHWA, Malakand Division**, Malakand district, 844 m a.s.l., on *Chenopodium ambrosioides* L. (*Amaranthaceae*), 19 September 2017, asexual morph, A. Anwar AA#4 (PUP Bot.01; GenBank MN368297); Upper Dir district, 844 m a.s.l., on *C. ambrosioides*, 29 October 2017, asexual and sexual morphs, A. Anwar AA#5 (PUP Bot.02); **Hazara Division**, Abbottabad district, Ayubia National Park, 2438 m a.s.l., on *C. ambrosioides*, 30 October 2017, asexual morph, N.S. Afshan NSA#34 (LAH 35658).

Phylogenetic results

The NCBI BLASTn analysis showed that our ITS sequence (MN368297 *Erysiphe betae)* closely matched (99.54% identity) a sequence of *E. betae* (LC009946) on *Beta vulgaris* from Japan (Takamatsu & al. 2015). The 20 nucleotide sequences used in the phylogenetic analysis included *Phyllactinia angulata* (AB080464) and *P. leveilluloides* (LC108847) as outgroup. The final aligned data set contained 818 positions of which 567 were conserved, 202 variable and parsimony uninformative, and 49 parsimony informative sites. Maximum likelihood (ML) analysis clustered the Pakistan *E. betae* collection (MN368297) with the Japanese *E. betae* collection (LC009946) with a 71 bootstrap value. Based on these results, we assigned the name *Erysiphe betae* to the powdery mildew pathogen on *Chenopodium ambrosioides* in Pakistan.

Discussion

Worldwide, three *Erysiphe* spp. (including *E. betae*), two *Leveillula* spp., and three anamorphic powdery mildew species have been reported on *Chenopodium ambrosioides* (Fungus-Host Database https://nt.ars-grin.gov, 29 May, 2018, Braun & Cook 2012). From Pakistan, only "*Erysiphe communis* (Wallr.) Schltdl." (nom. rej.) on *Chenopodium botrys* and *Spinacia oleracea* and *Leveillula cylindrospora* U. Braun and *L. taurica* (Lév.) G. Arnaud on *Chenopodium murale* L. have been reported. *Chenopodium ambrosioides* is reported here as a new host record for *Erysiphe betae* in Pakistan.

Previously, only asexual morphs of *Erysiphe* have been reported on *Chenopodium* species from Asia and Europe, whereas sexual fruiting bodies (chasmothecia) have been reported only from India and Japan (Braun & Cook 2012). We now report both asexual and sexual morphs of *Erysiphe betae* from Pakistan. We found only the asexual morph in the Abbottabad and Malakand districts, but additionally collected the chasmothecia in the Upper Dir district. Previous reports from Pakistan of *Erysiphe* on *Chenopodium botrys* (as "*E. communis*;" Amano 1986) is probably allocable to *E. betae*. Francis & al. (2007), who analysed ITS rDNA sequences retrieved from European (UK) and North American (USA) collections of *E. betae* on sugar beet, confirmed the involvement of a single recognizable *Erysiphe* species more closely allied to *Erysiphe heraclei* DC. than to *E. polygoni* DC. Takamatsu & al. (2015) included in their phylogenetic analyses a sequence obtained from *E. betae* on *Beta vulgaris* L. with corresponding results, but sequences retrieved from *E. betae* on *Chenopodium ambrosioides* are not yet available and still in need of molecular confirmation. Our research represents the first phylogenetic analysis of *E. betae* on *Chenopodium ambrosioides*, which clusters our ITS sequence in a strongly supported clade with a sequence of *E. betae* on *Beta vulgaris* (Fig. 1).

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Crustose lichens new to India

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Abstract—*Bacidia pycnidiata* (*Ramalinaceae*), *Malmidea nigromarginata* (*Malmideaceae*), *Porina malmei* and *P. nuculastrum* (*Porinaceae*), and *Pyrenula laetior* and *P. wrightii* (*Pyrenulaceae*) are reported for the first time from India. The specimens were collected from the state of Assam. Taxonomic descriptions, distributions, and illustrations are provided for each species.

Key words—biodiversity, lichenized *Ascomycota*, Nagaon, taxonomy, Tezpur

Introduction

Lichens constitute a significant part of India's biodiversity, and 2714 lichen species are known from the country (Sinha & al. 2018). Although lichens occur everywhere in India, ecological studies reveal that Northeast India, Western Ghats, Western Himalaya, and Andaman & Nicobar Islands are the major centres of diversity (Nayaka & Asthana 2014). Assam, a state of Northeast India, possesses the heterogeneous climatic conditions that make it suitable for rich floral, faunal, and fungal diversity. Over 325 species (in 83 genera and 27 families) have been reported from Assam (Gupta & Sinha 2018, Gogoi & al. 2019). Here we record six additional lichen species (in *Malmideaceae*, *Porinaceae*, *Pyrenulaceae*, and *Ramalinaceae*) new to Assam and to India.

Materials & methods

The present study is based on the freshly collected lichen samples from Assam. The lichen specimens were dried, preserved, and deposited in the herbarium at CSIR-National Botanical Research Institute, Lucknow, India (LWG). The lichen thallus was morphologically examined under a Leica EZ4 stereo zoom microscope. Thin hand-cut sections of ascus and thallus were mounted in distilled water, Lactophenol Cotton Blue (LCB), 5% KOH, or Lugol's iodine solution for observation under a Leica DM 2500 compound microscope. The thallus and ascomatal tissue were spot-tested with K (5% KOH aqueous solution), C (aqueous Ca(ClO)2 solution) and P (0.5 g of para-phenylenediamine dissolved in 5 ml of ethanol) following Orange & al. (2001). Thin Layer Chromatography was performed in solvent system C (toluene: acetic acid; 85: 15 ml) following Orange & al. (2001).

Taxonomy

Bacidia pycnidiata Czarnota & Coppins, Lichenologist 38: 407. 2006 Pl. 1a

Thallus epilithic, pale yellowish to greenish, slightly granular, cracked. Apothecia few, 0.2–0.4 mm diam. Excipulum prominent, colourless, 90–140 µm wide. Hymenium hyaline, 40–55 µm high, I+ blue. Asci cylindrical, *Bacidia*-type, 35–41 × 4.5–5.8 µm. Ascospores acicular, 3(–7)-septate, 25–38 × 1.2–2.8 µm. Pycnidia very few, having round flask shaped bottom with long neck, 0.1–0.2 mm diam., constricted at base, concolorous with apothecia, neck slightly paler than bottom, ostiole present at the tip of the beak; conidia hyaline, 3–5-septate, straight to slightly curved, filiform, 37–62 × 0.4–1.0 µm.

Chemistry—Thallus K–, C–, P–, UV–; no chemicals detected in TLC.

Specimen examined—**INDIA**, **Assam**, **Tezpur**, Ouguri Hills, 26°37′03″N 92°46′37″E, elev. 113 m, on rock, 16 May 2015, Manas P. Choudhury 15027001 (LWG 35885).

Remarks—The ascospore length of the Indian specimen is slightly shorter than that given in the protologue of *Bacidia pycnidiata* (35–52 µm; Czarnota & Coppins 2006). Further, the Indian specimen had occasional 7-septate ascospores rather than the usual 3-septate, which has not been reported previously for *B. pycnidiata*. In all other aspects the Indian specimen agrees with the published description of *B. pycnidiata*.

Distribution—Belgium, Czech Republic, Estonia, Finland, Lithuania, Mordovia, North Caucasus, Poland, and Ukraine (Urbanavichene & Urbanavichus 2014). This is a first report of *B. pycnidiata* for India.

Malmidea nigromarginata (Malme) Lücking & Breuss, Lichenologist 47: 19. 2015. Pl. 1b

Thallus crustose, epiphloeodal, smooth, grayish black, medulla ochraceous, K+ yellow; borderline blackish. Apothecia mostly solitary,

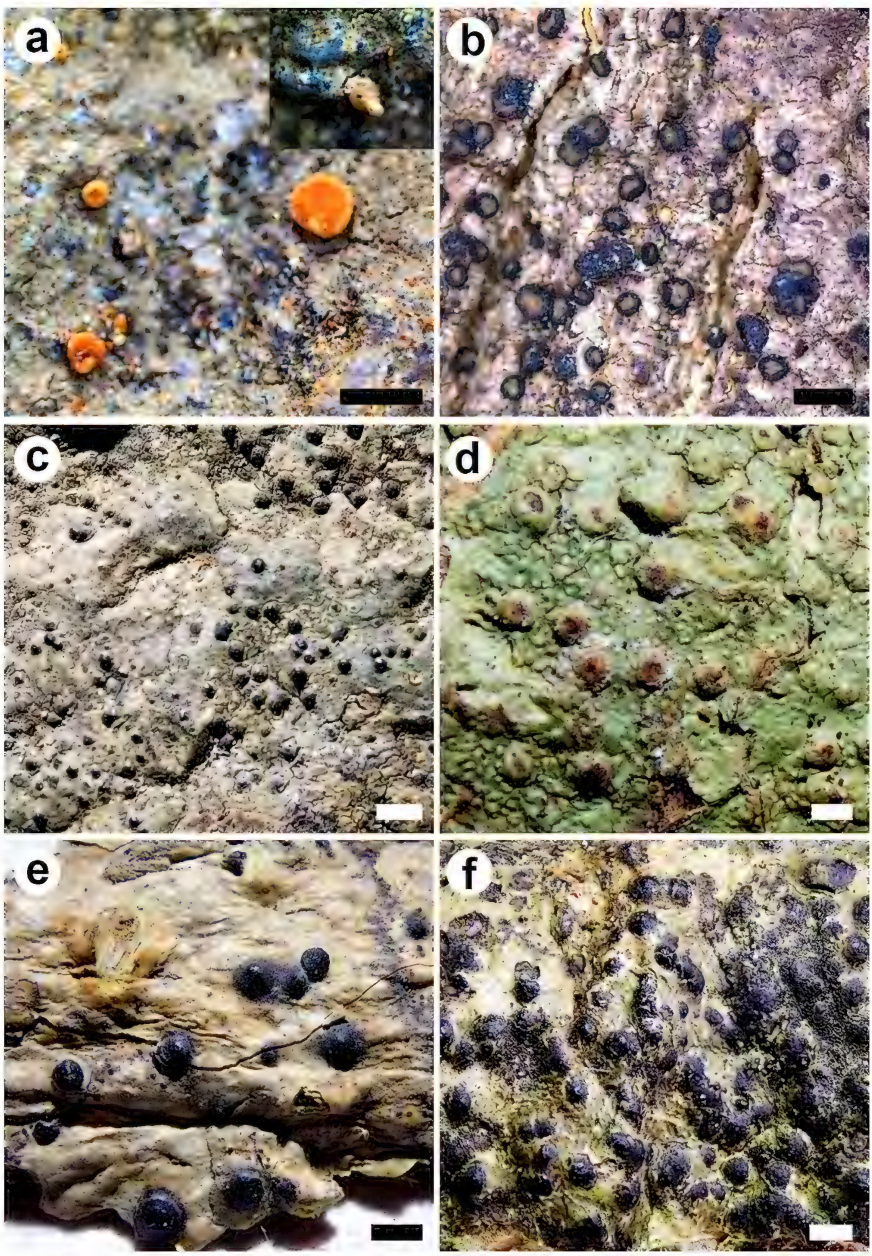

Plate 1. Habit. a. ***Bacidia pycnidiata*** (LWG 35885); b. ***Malmidea nigromarginata*** (LWG 35887); c. ***Porina malmei*** (LWG 35886); d. ***Porina nuculastrum*** (LWG 35888); e. ***Pyrenula laetior*** (LWG 35889); f. ***Pyrenula wrightii*** (LWG 35890). Scale bars = 1 mm.

rarely 3–4 aggregated, margin black, disc flesh coloured. Excipulum without algal cells, lacking medullary layer, hyphae radiating and conglutinated. Hymenium hyaline, 70–90 µm high, I+ blue. Hypothecium dark brownish to brown black, 70–100 µm thick. Asci 6–8-spored. Ascospores simple, hyaline, 8.9–13.4 × 4.5–5.4 µm.

Chemistry—Thallus K–, C–, P–, UV–; no lichen substances detected in TLC.

Specimen examined—**INDIA**, **Assam**, **Nagaon district**, Samaguri, Chapanalla, 26°19′13″N 92°54′16″E, elev. 119 m, on bark, 18 February 2018, Rupjyoti Gogoi NCLH 030 (LWG 35887).

Remarks—The ascospore sizes of the Indian specimen fit well within the range of *Malmidea nigromarginata* (10–14 × 4–6 µm) as mentioned in the world key to the genus by Breuss & Lücking (2015).

Distribution—Nicaragua (Breuss & Lücking 2015) and Puerto Rico (Mercado-Díaz & al. 2015). This is a first report of *M. nigromarginata* for India.

Porina malmei P.M. McCarthy, Biblioth. Lichenol. 52: 70. 1993. Pl. 1c

Thallus saxicolous, medium to dark grey, areolate, ecorticate. Perithecia emergent, hemispherical to subglobose, dull black, 0.1–0.3 mm diam. Ostioles apical, conspicuous. Involucrellum brownish, continuous with the exciple. Excipulum black, 20–50 µm thick. Centrum 0.15–0.25 µm wide. Hymenium clear. Asci elongate-cylindrical. Ascospores hyaline, fusiform, (3–)6–7-septate, 20–27 × 4–6 µm. Pycnidia not seen.

Chemistry—Thallus K–, C–, P–, UV–; no chemicals detected in TLC.

Specimen examined—**INDIA**, **Assam**, **Tezpur**, Rudrapad Temple, bank of River Brahmaputra, 26°36′55″N 92°46′10″E, elev. 65 m, on rock, 16 March 2015, Manas P. Choudhury 15027003 (LWG 35886).

Remarks—The Indian specimen agrees with the description of Australian *Porina malmei* in most features, and ascospore lengths fit well within the 17–30 µm range cited in the protologue (McCarthy 1993).

Distribution—Brazil, north-eastern Australia (McCarthy 2000). This is a first report of *P. malmei* for India.

Porina nuculastrum (Müll. Arg.) R.C. Harris, More Florida Lichens, incl. 10 Cent Tour Pyrenol. (New York): 174. 1995. Pl. 1d

Thallus crustose, epiphloeodal, olivaceous, slightly rimose, prothallus black. Perithecia erumpent to ± immersed, mostly solitary, rarely grouped into 2–3, perithecia concolorous with the thallus except in the peri-ostiolar and ostiolar regions, convex to sub-globose 0.4–0.65 mm diam.; ostiole inconspicuous or

in a shallow depression; peri-ostiolar area 0.12–0.18 mm diam. Involucrellum enclosing the exciple yellowish to blackish. Exciple ca. 30 µm thick. Hymenium clear. Asci elongate-cylindrical. Ascospores narrowly ellipsoidal to fusiform, submuriform to muriform with 6–10 transverse septa and (0–)1(–3) longitudinal septa, 49.8–67.6 × 13.5–22.2 µm. Pycnidia not seen.

Chemistry—Thallus K–, C–, P–, UV–; no chemicals detected in TLC.

Specimen examined—**INDIA**, **Assam**, **Nagaon district**, Samaguri, Suang Reserve Forest, 26°19′20″N 92°54′18″E, elev. 142 m, on bark, 18 February 2018, Rupjyoti Gogoi NCLH 031 (LWG 35888).

Remarks—The Indian specimen closely matches the Australian and Vietnamese descriptions of *Porina nuculastrum* (McCarthy 2001, Joshi & al. 2019), except that our ascospores have fewer transverse septa.

Distribution—Neotropics, Madagascar, the Philippines, Hong Kong and neighbouring countries (McCarthy 2001), Vietnam (Joshi & al. 2019). This is a first report of *P. nuculastrum* for India.

Pyrenula laetior Müll. Arg., Bot. Jahrb. Syst. 6: 413. 1885. Pl. 1e

Thallus crustose, corticate, epiphloeodal, yellow, without external pigments. Perithecia black, solitary, 0.4–0.9 mm diam. Ostiole apical without pigment. Hamathecium inspersed with oil droplets. Asci 8-spored. Ascospores brown, 3-septate, eciliate, distoseptate, lumina in a straight line, 13.8–19.2 × 5.8–7.7 µm, central lumina not strongly elongated, terminal lumina separated from the exospore wall by endospore thickenings, old ascospores without orange oil.

Chemistry—Thallus K–, C–, P–, UV–; no chemicals detected in TLC.

Specimen examined—**INDIA**, **Assam**, **Nagaon district**, Samaguri, Suang Reserve Forest, 26°19′20″N 92°54′18″E, elev. 143 m, on bark, 18 February 2018, Rupjyoti Gogoi NCLH 032 (LWG 35889).

Remarks—Aptroot (2012) cites ascospores ranging from 13–18 µm for *Pyrenula laetior,* while those of the Indian specimen are slightly longer. The Indian material agrees with *P. laetior* in all other characters.

Distribution—Neotropics (Aptroot 2012), Vietnam (Joshi & al. 2018). This represents a first report of *P. laetior* for India.

Pyrenula wrightii (Müll. Arg.) R.C. Harris,
More Florida Lichens, incl. 10 Cent Tour Pyrenol.: 111. 1995. Pl. 1f

Thallus corticolous, epiphloeodal, olive green with pseudocyphellae. Perithecia black, without external pigments, solitary to mostly aggregated with separated ostioles, 0.2–0.5 mm diam. Ostiole apical without pigment.

Hamathecium not inspersed with oil droplets. Asci 8-spored. Ascospores brown, 2–3-septate, 27.3–33.2 × 12.5–14.5 µm, lumina in a straight line, external lumina separated from the exospore wall by endospore thickening.

Chemistry—Thallus K–, C–, P–, UV–; no lichen substance detected in TLC.

Specimen examined—**INDIA, Assam, Nagaon district**, Doboka Circle, Jamunamukh, 26°05′54″N 92°44′44″E, elev. 40 m, on bark, 5 February 2017, Rupjyoti Gogoi NCLH 033 (LWG 35890).

Remarks—The Indian specimen agrees with the published description of *Pyrenula wrightii* by having pseudocyphellae and in ascospore characteristics (Aptroot 2012). The ascospores of the Indian specimen are slightly longer than the measurements provided in the basionym protologue (27–30 µm; Müller 1885).

Distribution—Cuba (Aptroot 2012). This represents a first report of *P. laetior* for India and the first outside the type country.

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Chromelosporium re-evaluated, with *Chromelosporiopsis* gen. nov. and *Geohypha* stat. nov.

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Abstract—*Chromelosporium*-like asexual morphs of the subterraneous *Pezizaceae* in the /*Pachyphlodes* clade are not congeneric with the type of *Chromelosporium*. Some *Chromelosporium* morphs are of species in the /*Pezizaceae* clade (the /*Peziza* clade), and the genus *Chromelosporium* as it has been defined is polyphyletic. A diagnostic character that distinguishes these two groups is the presence or absence of conidiophore fasciculation. Mononematous conidiophores characterize *Chromelosporium* and species related to the /*Peziza* clade. Synnematous conidiophores characterize *Pachyphlodes* and other asexual species and define the new genus *Chromelosporiopsis,* to be excluded from *Chromelosporium*. *Hyphelia terrestris,* long misapplied to *Chromelosporium,* is revaluated, lectotypified, redescribed and recombined as *Geohypha terrestris*.

Keywords—hyphomycete, nomenclature, taxonomy

Introduction

Collections of *Chromelosporium*-like hyphomycetes made by R.P. Korf around Ithaca, NY, which he tentatively named *Glischroderma* because of their production of gel (Korf 1994), were revaluated by Healy & al. (2015) and identified as conidial morphs in the truffle genus *Pachyphlodes*. Fresh *Chromelosporium*-like specimens collected on bare soil in woods in Belgium, identified by DNA sequencing as *Pachyphlodes nemoralis* and *P. citrina,* provided the necessary asexual material to compare with and segregate from the asexually typified species, *Chromelosporium ochraceum* (Hennebert & Decock 2020).

The main distinction that emerged between the asexual morphs in *C. ochraceum* and the two *Pachyphlodes* species is conidiophore fasciculation. This criterion, applied to species already named in *Chromelosporium* (Hennebert 1973), segregates them into two genera. It seems generally accepted that these fungi are asexual morphs of *Pezizales*. As long as their type species are not known to be congeneric with species with a known sexual morph, however, it seems sensible to keep these genera separate.

Mononematous species define *Chromelosporium*. Synnematous species are excluded and are accommodated in the new genus *Chromelosporiopsis*.

Names such as *Botrytis epigaea*, *Phymatotrichum* species, and others are discussed as doubtful taxa. The conidial ornamentation observed in the numerous herbarium specimens that might contribute to the diagnosis of unidentified *Chromelosporiopsis* species is also analysed.

Hyphelia terrestris, currently misclassified in *Chromelosporium*, is re-evaluated and recombined as the type of *Geohypha* [≡ *Hyphelia* sect. *Geohypha* elevated to generic rank].

The asexual morph of *Plicaria endocarpoides*, previously supposed to show similarities with *Chromelosporium*, is described.

The illustrated descriptions of the asexual morphology of these taxa provide the rationale for the present paper.

Materials & methods

Species descriptions are based on observations made on herbarium material borrowed from many herbaria and some fresh collections made during 1960–62 while working as a postdoctoral researcher under the late Dr S.J. Hughes at the Agriculture & Agri-Food mycology group in Ottawa (DAOM). This material is complemented with some contemporary collections from Europe. A 1960 Olympus FH microscope equipped with Olympus positive low phase contrast objectives and an adapted Wild drawing tube was used for the microscopic examinations. All samples were mounted in lactic acid with Cotton blue. Slides made from the examined specimens are preserved in the Canadian National Mycological Herbarium, Ottawa, Canada (DAOM) and/or Mycothèque, Université Catholique de Louvain, Louvain-la-Neuve, Belgium (MUCL), and accession numbers are listed for each specimen in square brackets. Information provided in the Examined specimen sections duplicates the herbarium labels with bold numbers separating each specimen.

Taxonomy

Chromelosporium

This section includes the mononematous species considered morphologically congeneric with the type species *Chromelosporium ochraceum*, which currently

delimits the genus *Chromelosporium*. In addition, one *Peziza* and one *Plicaria* species with similar asexual morphs are each described.

Chromelosporium Corda, in Sturm's Deutschl. Fl., Abt. 3 (Pilze), 3(13): 81, 1833.

Type species: *Chromelosporium ochraceum* Corda??

Ascomycota, *Pezizaceae*, sexual morph unknown.

Hyphae septate, creeping on the substratum.

Conidiophores mononematous, erect, septate, apically branching in successive symmetrical dichotomies, septate, apically obsuse.

Conidiogenus cells subterminal and terminal branches forming synchronous conidia on denticles along their lengths, collapsing after rhexolytic secession of conidia.

Conidia holoblastic, one-celled, globose or subglobose, with wall ornamented from finely to coarsely verrucose, colored in mass.

Habitat: on plant fragments.

Comments—Hennebert (1973) made a clear distinction between *Chromelosporium* and *Ostracoderma* Fr., so *Chromelosporium* should not be placed as a synonym of *Ostracoderma* as cited in the 10th edition of Ainsworth & Bisby's Dictionary of the Fungi (Kirk & al. 2008) and in Index Fungorum. *Chromelosporium* is also incorrectly listed as a synonym of *Peziza* in Baral's contribution to Jaklitsch & al. (2016). Such synonymy is indeed impossible—*Peziza* being clearly polyphyletic and *Chromelosporium* an "orphan" genus. Pfister & al. (2016) did not mention the case when evaluating competing sexually and asexually typified names. Indeed, as the type species is not connected to any sexual morph, the generic name *Chromelosporium* cannot be competing so far. Also, *Chromelosporium* should not have been ignored in the recent "Outline" of fungal genera (Wijayawardene & al. 2020). It is possible that the four species described in the genus below, beside the type species, might also be revealed as polyphyletic.

Chromelosporium ochraceum Corda,

in Sturm's Deutschl. Fl., Abt. 3 (Pilze), 3(13): 81, 1833. Figs 1, 2

≡ *Sporotrichum ochraceum* (Corda) Sacc. Syll. Fung. 4: 105, 1886.

≡ *Ostracoderma ochraceum* (Corda) S. Hughes, Canad. J. Bot. 36: 792, 1958.

= *Botrytis dichotoma* Corda, Icon. Fung. 1: 18, 1837.

≡ *Campsotrichum dichotomum* (Corda) Bonord., Handb. Mykol.: 102, 1851.

= *Botrytis isabellina* Preuss, Linnaea 25: 75, 1852.

≡ *Ostracoderma isabellinum* (Preuss) S. Hughes, Canad. J. Bot. 36: 792, 1958.

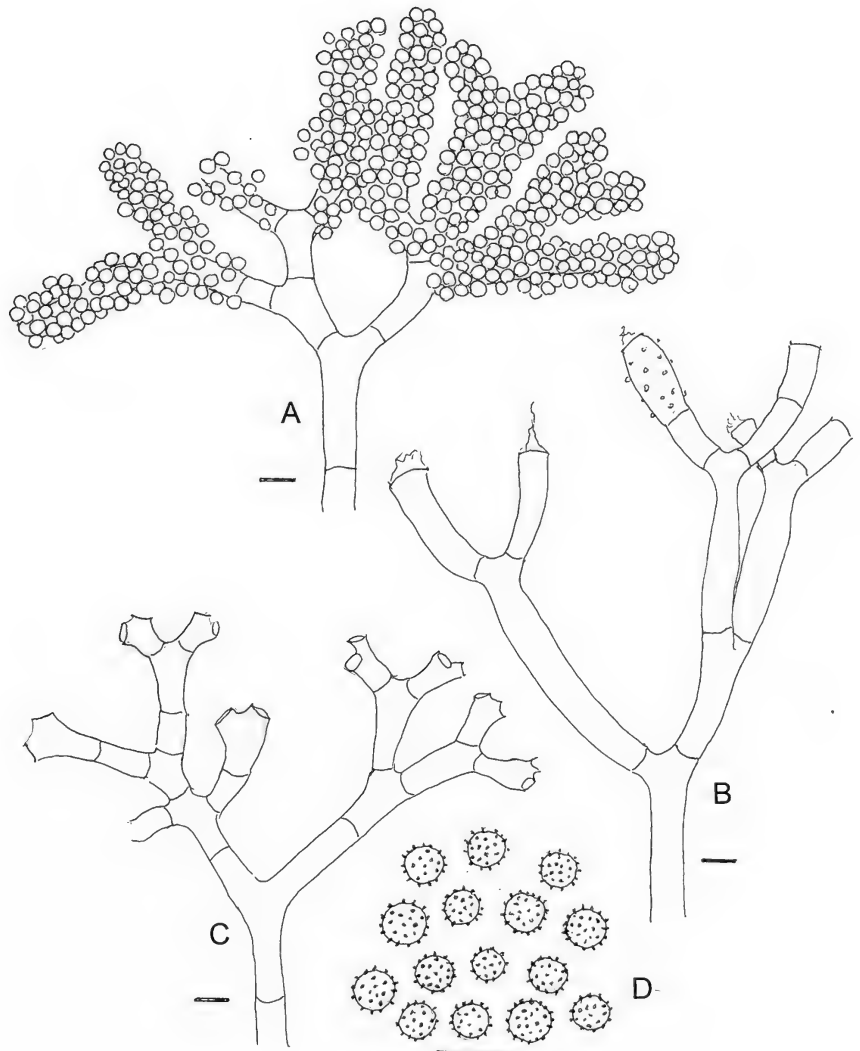

Fig. 1. *Chromelosporium ochraceum*. A. Conidiophore with conidiogenous cells (drawing by S.J. Hughes, DAOM 83330). B, D. Conidiophore and conidia (holotype, PRM 155414), C. conidiophore after secession of the conidiogenous cells (DAOM74695, Mycoth. Ven. 1080). Scale bar = 10 µm.

= *Polyactis crystallina* Bonord., Abh. Naturf. Ges. Halle 8: 95, 1864.

≡ *Botrytis crystallina* (Bonord.) Sacc., Syll. Fung. 4: 135, 1886.

= *Botrytis spectabilis* Harz, Bull. Soc. Imp. Nauralistes Moscou 44(1): 144, 1871.

= *Clonostachys spectabilis* (Harz) Oudem. & Sacc., Ned. Kruid. Arch., 2. ser., 4: 539, 1886.

Type: *Chromelo~~trichum~~* [crossed out] *sporium ochraceum* Ca. *ramulis furcis, Myrinema* generis" [scr. et del. A.C.J. Corda]. On *Allium* dead stem, Prague, Czechoslovakia. Herb. Corda (**Holotype:** PRM 155414; **isootypes:** [DAOM 40946, MUCL.2895]

Colonies in small patches, ≤10 mm diam., velvety, at first white, then ochraceous when mature.

Hyphae 3–5 µm wide, creeping in the substratum.

Conidiophores mononematous, erect, emerging from hyphae in substratum, with a bulbous basal cell, 28–35 × 12–15 µm, ochraceous, extending into a cylindrical, septate stipe, 100–400 µm long, pale ochraceous, the individual cells 40–95 × 10–14 µm, terminally repeatedly branched with up to five dichotomies, each branch with a basal septum and a second septum before the subsequent dichotomy, the length of branch decreasing from one dichotomy to the next, usually the first dichotomy 40–90 × 9–10 µm, the second 30–70 × 6–10 µm, the third 30–50 × 6–9 µm and so on, but in some cases dichotomies being very short decreasing from 20 to 10 µm, the angle of the dichotomies ranging from 30° to 45°.

Conidiogenous cells comprising the last four and five terminal dichotomies, together 70–100 × 6–9 µm, apically clavate, ≤11 µm, covered with synchronous conidia borne on a denticle, and collapsing after conidial release, seceding or (rarely) remaining as a frill on the last conidiophore cell.

Conidia holoblastic, borne simultaneously on denticles 2 × 1 µm, one-celled, globose or subglobose, ochraceous to salmon-colored in mass, 4–6.5 µm, wall 0.5 µm thick, cyanophilic, verrucose, warts prominent 0.3 µm high and blunt, with 12–14 warts in median view, seceding rhexolytically.

Habitat: on plant fragments.

Distribution: Europe: Italy, France, Belgium, England, Africa: Maroc, Uganda, Asia: Sumatra.

Comments—The name *Chromelosporium* remained unused until Hughes (1958) brought the genus to light. Corda's 1833 description of an overmature collection was so unclear that even Corda (1837) himself did not recognize the conspecificity of his later taxon, *Botrytis dichotoma*. His figure (Fig. 3A) shows globose ochraceous conidia accumulated along the sides and the top of the obtuse dichotomous branches.

Korf & Hennebert (1975) stated that *Botrytis spectabilis* was a synonym of *Chromelosporium ochraceum* based on an original illustration from Harz reproduced here (Figs. 3B-C) and preserved in MUCL 1072.

The names *Botrytis spectabilis* and *Polyactis crystallina* (another synonym of *Chromelosporium ochraceum*) have been misapplied to the asexual morph

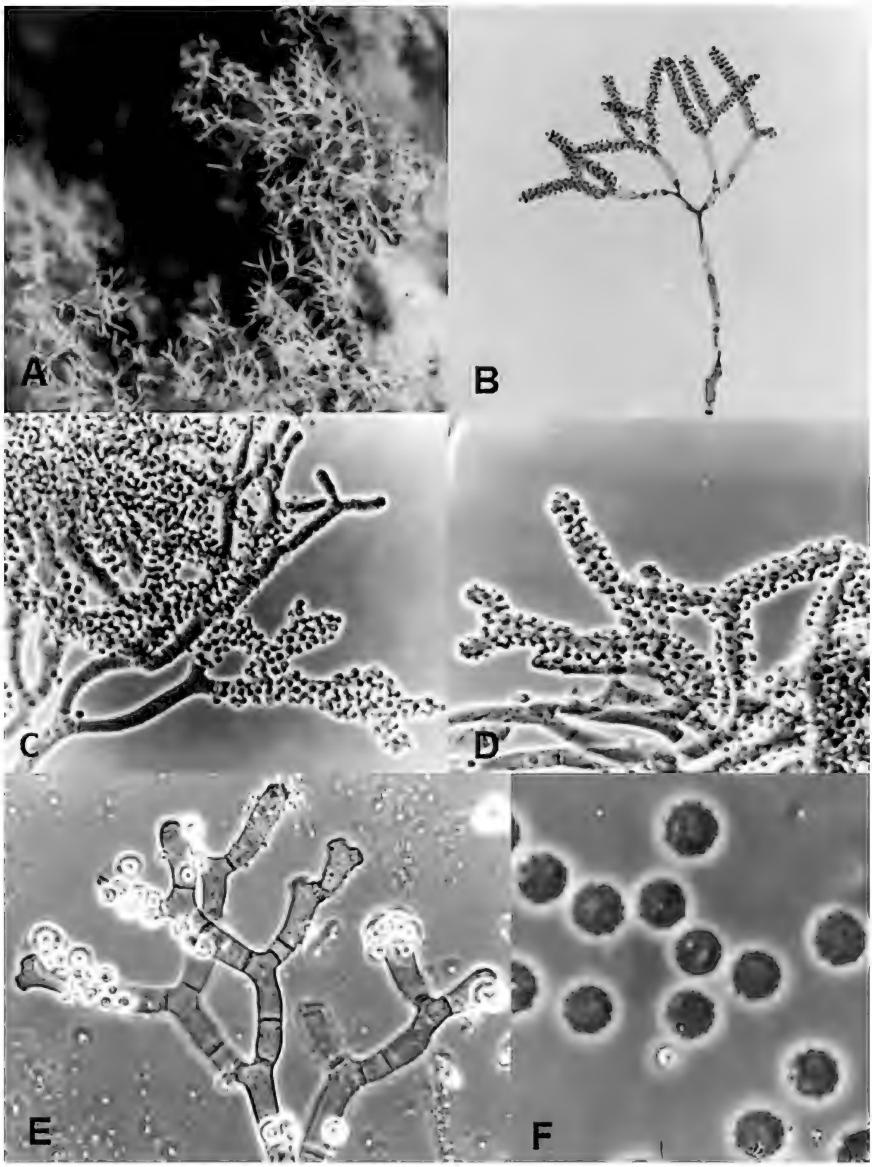

Fig. 2. *Chromelosporium ochraceum* (DAOM 46357). A. Conidiophores under low magnification. B. Dichotomies of conidiophore. C-D. Conidiogenous dichotomies. E. Conidiophore after secession of conidiogenous cells. F. Verrucose conidia.

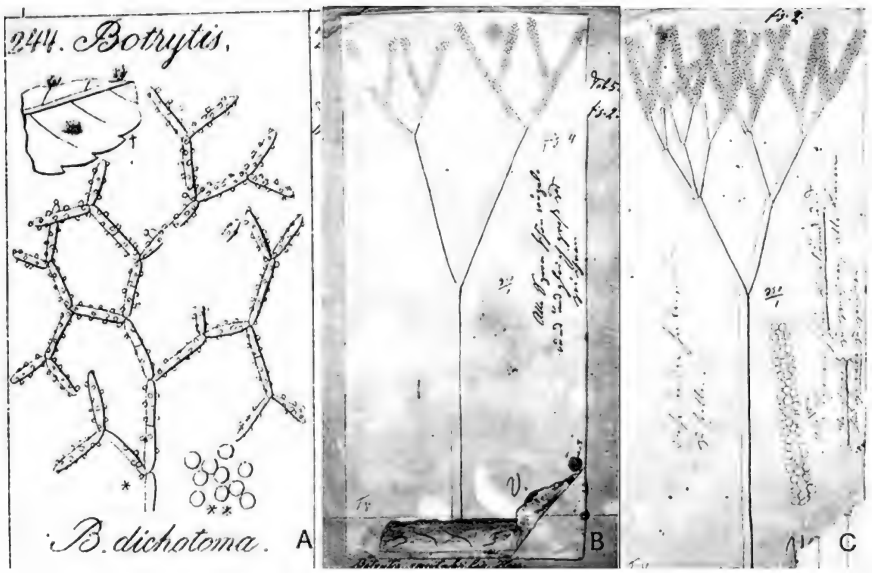

Fig. 3. Illustrations of A. ***Botrytis dichotoma*** Corda, Icon. Fung. 1: 18, fig. 244, 1837. B-C. ***Botrytis spectabilis*** Harz, original drawings (MUCL 1072).

of *Peziza ostracoderma* Korf (Hennebert & Korf 1975). For that reason, both *Botrytis spectabilis* and *Polyactis crystallina* have been erroneously listed as synonyms of *Peziza ostracoderma* in Index Fungorum. *Chromelosporium ochraceum* is also erroneously cited as a synonym of *C. fulvum* (Link) McGinty& al., typified by the asexual morph of *Peziza ostracoderma* in Seifert & al. (2012, p. 139).

The lectotype collection of *Botrytis isabellina* and the authentic collection distributed in Rabenhorst's Klotzschii Herbarium vivum mycologicum n° 1570, were described in sufficient detail by Preuss (1853) as "Floccis erectis crassis repetito ramosis dichotomis vel trichotomis, apice verrucosis sporis irregulariter accumulatis, globosis, hilo instructo, episporio verrucoso" to confirm its synonymy with *Chromelosporium ochraceum*.

Specimens examined

Type & authentic material. **EUROPE: Czechia:** (2) ***Botrytis dichotoma,*** Herb.Corda (PRM 155393 type), [DAOM 84706, MUCL 2896]. **Germany:** (3) ***Botrytis isabellina*** Preuss 1850, auf [rotten] nadelholz, Herb Preuss 451 (B) **(designated here as lectotype MBT 392406)** [MUCL 5999]. (4) *Botrytis isabellina* Pr., ad lignum abietinum humi jacentem, prope Hoyerswerda [Germany], Preuss [1851],

Rabenh., Klotzschii Herb. Viv. Mycol. 1570 (B, PR, BM, BR, PAV, L) (isolectotypes) [DAOM 51762, MUCL 1810]. (5) ***Botrytis spectabilis*** Harz, original drawing by Harz (isolectotype 1975) (MUCL 1072).

Other specimens. **EUROPE**: **Italy:** (**6**) *Botrytis epigaea* Link f. *ochracea* Sacc, ad terram argillosam in horto botanico, Padova. Aug. 1902. D. Saccardo, Mycotheca italica Mucedinaceae. [1903, Cent. XII] n° 1178 (PR 184863, BM, L 910.224-158) [DAOM 74898, MUCL 1803]. [The name has been listed in Michelia I :102, 1877]. (**7**) *Botrytis dichotoma* Corda, in sarmentis, foliis, capsulis putrescentibus, in Horto botanico ticinensi. Autumno, coll. Cavara. Micol. Lomb. n° 137, tab. 1, fig. 20-24 [1894]. Cavara, Fungi Longobardiae exsiccate. 195, (PAD, BM, PAV, BPI, NY, B, L) [DAOM 83330, MUCL 1801]. (**8**) *Botrytis fulva* Link. in scapis *Allii cepae*, Consiglio (Treviso). Sept. 1897. D. Saccardo Mycotheca italica Mucedinaceae. Cent. II.186, (K-M) [DAOM 74699, MUCL 1802]. (**9**) *Botrytis fulva* Lk. *Polyactis fulva* Bon. in truncis putridis. Selva (Treviso) Oct. 1876. Saccardo P.A. Mycotheca veneta. Cent. XI. 1897. n°1080 (K-M) [DAOM 74695, MUCL 1812]. (**10**) *Botrytis fulva* Link, ad corticem ramorum, Valtaro, Sept. 1923, coll. Renji, det. G. Bresadola, (BPI) [DAOM 83884, MUCL 2413]. (**11**) *Botrytis fulva* Link f. *argillacea,* ad *Salicem petandram*, Eichler, Polachia, 1894. [scr. Bresadola] Herb. Bresadola, [In Bresadola Fungi polonici a cl. Viro B. Eichler lecti. Ann. Mycol. 1, 2: 97-131, p. 127(1903), forma *argillacea* of *Botrytis fulva* Link is not described] (S) [MUCL2531]. **UK**: (**16**) *Botrytis fulva* Link. Queen's Cottage grounds, Kew, Nov. 1898. G.E. Massee. (K-M) [MUCL 2295]. (**17**) *Phymatotrichum*, on *Fagus sylvatica* rotten wood, Sheepleas, Horsley, Surrey, R.W.G. Dennis, March 2 1952. (K-M–IMI 49481) [MUCL 3547]. (**18**) *Botrytis splendida* Schwein., on mossy *Salix* bark, Wheatfen Broad, Norfolk, E.A. Ellis , (K-M–IMI 26983) [MUCL 3568]. (16) *Phymatotrichum*, on *Petasites ovalis*, Forge Valley, Yorkshire, coll. I.M.I., det. S.J. Hughes. (K-M–IMI 8160) [MUCL 3581]. (**19**) *Botrytis (Phymatotrichum)*, on *Heracleum*, Forge Valley, Yorkshire, Sept. 16 1946, coll. I.M.I., det. S.J. Hughes. (K-M-IMI 6851) [MUCL3592]. (**20**) *Botrytis*, *Phymatotrichum*, on *Petasites* dead petioles, Forge Valley, Yorkshire, Oct. 1945, coll. I.M.I., det. S.J. Hughes, (K-M–IMI 1586) [MUCL 3595]. (**21**) *Phymatotrichum*, on *Eupatorium cannabinum*, Farwath, Newton Dale, Pikering, Yorkshire, Aug. 6 1960, W. Bramley. (K-M–IMI 83016a) [MUCL 3622]. (**22**) *Phymatotrichum*, on *Heracleum*, Forge Valley, Yorkshire, Sept. 16 1946, coll. I.M.I., det. S.J. Hughes. (K-M–IMI 7341) [MUCL 3582]. **France**: (**12**) *Botrytis fulva* Link, in ligno putrido, Côte d'Or, Sept. 1889, coll Fautrey n°9 (PC) [DAOM 83390, MUCL 2378]. (**13**) *Botrytis fulva* Bon., sur tricot de coton pourri près d'un lavoir, Lyon, coll. J.J. Therry. Cryptogames du Lyonnais. J.J. Therry n° 6474, (G) [MUCL 2398]. (**14***) Botrytis carnea* Schum., ad culmos Paludi, Meudon, Nov. 3 1893, coll. E. Roussel]. (PC) [MUCL 2706]. **Belgium**: (**23**) *Phymatotrichum*, on herbaceous stems and leaves of compositae, Forêt de Soignes, Sept. 16 1956, coll. M.B. Ellis. (K-M–IMI 69956) [MUCL 3542].

AFRICA: **Morocco:** (**24**) *Phymatotrichum*, sur souche de *Quercus ilex*, Forêt d'Azrou, Moyen Atlas, Maroc, 17 Nov. 1965, G. Malençon. Herb. Cryptog. G. Malençon 5805, (PC) [MUCL 8041]. **UGANDA**: (**25**) *Botrytis*, on dead wood, Kampala, 4000', May 1936, G. Hansford. Herb. Mycol. Dep. Agric. Uganda n° 1769 (K-M–IMI 7899) [MUCL 3613].

ASIA: Indonesia: (**26**). *Botrytis*? op aarde, bladeren, takjes, Sumatra, Kampong Baroe bij Medan, Juli 1927, K.B. Boedijn. n° 4619 (Bot. Museum Utrecht 179892) [MUCL 5381].

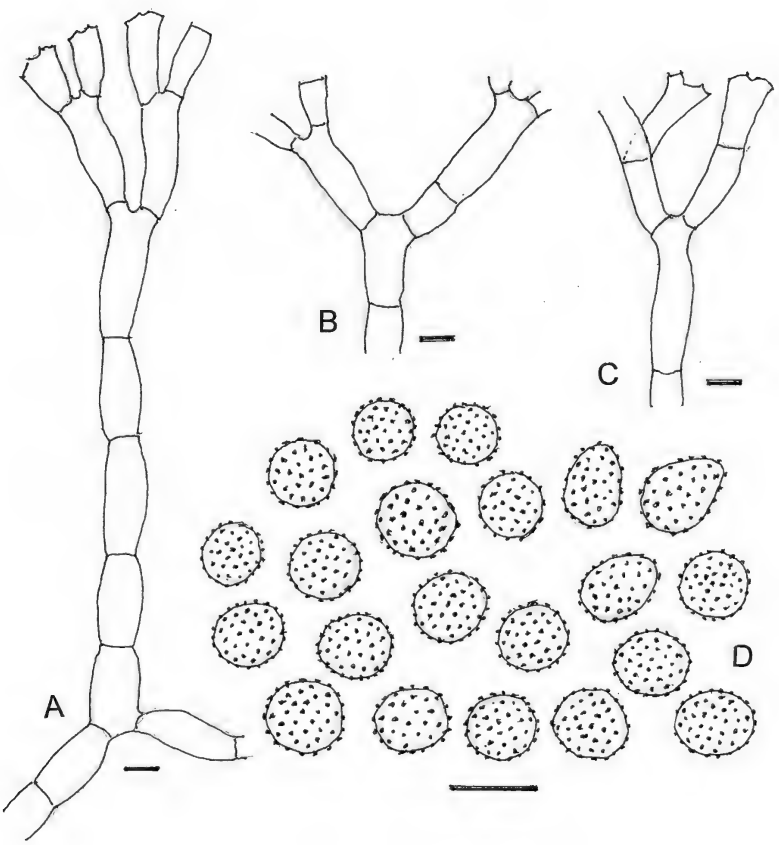

Fig. 4. *Chromelosporium herbicola* (isotype NY, MUCL 5889). A-C. Conidiophores missing the seceded conidiogenous cells. D. Verrucose conidia. Scale bar = 10 µm.

Chromelosporium herbicola (Ellis & Dearn.) Hennebert, **comb. nov.** Fig. 4

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≡ *Rhinotrichum herbicola* Ellis & Dearn., Proc. Canad. Inst., n.s. 1: 90, 1897 [(as "*herbicolum*"].

Type: *Rhinotrichum herbicolum* E[llis] & Dearness, on *Solidago virga-aurea* golden rod stems, Aug. 13 1895, D.V.S. Valley, London, Canada, J. Dearness. Herb. Dearness 2314 (**Holotype:** DAOM [MUCL2321]; **isotype** NY [MUCL 5889]).

Colonies in small patches, 3–5 mm wide, about 1 mm high, ochraceous.

Hyphae 5–15 µm wide, creeping on the substratum.

Conidiophores mononematous, erect, emerging single from creeping hyphae, as a stipe 100–150 µm high, 9–15 µm wide, ochraceous, slightly constricted at septa in cells 25–35 µm long, terminally branching in successive dichotomies, at 10–45° angles, branches septate at the base and sometimes over their length, first dichotomies 30–50 µm long, second dichotomies 20–25 µm long,

Conidiogenus cells presumably the third and fourth dichotomies collapsed and fallen away, not observed in the material but similar to those of *Chromelosporium fulvum* after Sumstine (1911).

Conidia presumably holoblastic, borne simultaneously all along the conidiogenous cell as described by the original authors, globose to obovate, one-celled, (6–)7–9(–11) µm, ochraceous, thick-walled and verrucose, with 18–20 warts in median view, seceding rhexolytically.

Habitat: On decaying herbaceous plant stems, similar to *Solidago.*

Distribution: Canada: Ontario.

Comments – The original description of *Rhinotrichum herbicola* by Ellis & Dearness reads: "Effuse, light yellow, becoming brown in the center. Hyphae coarse, septate, branched, nearly hyaline, 8–10 µ, thick, Fertile hyphae sub-undulate above, tips swollen and bearing the globose, sub-hyaline, finely echinulate, 7–9 µ, conidia. On dead stems of *Solidago canadensis*, London, Can., Aug. 1895. Herb. Dearness n°2314."

Sumstine (1911) examined the isotype specimen in NY and observed that the "spores clustered at the apex of the fertile branches" Although "the specimen proved to be poor", he concluded that "it resembles in many respects *Botrytis fulva* Link." This comment joined to the observed dichotomous branching and the verrucose globose conidia leads to transfer the species to *Chromelosporium*.

The species differs from *Chromelosporium ochraceum* by its shorter conidiophores and conidial size and ornamentation.

Chromelosporium canadense Hennebert, Persoonia 7: 196, 1973. FIGS 5, 6

= *Rhinotrichum carneum* Ellis & Everh., J. Mycol. 1(7): 93, 1885
(non *Chromelosporium carneum* (Pers.) Hennebert

Type: *Chromelosporium canadense* Hennebert, on rotten wood in forest, Gatineau Park, Gatineau, Quebec, Canada, 25.11.1960, leg. S.J. Hughes & G.L. Hennebert, (**Holotype:** DAOM 71947; **isotype:** MUCL 1689).

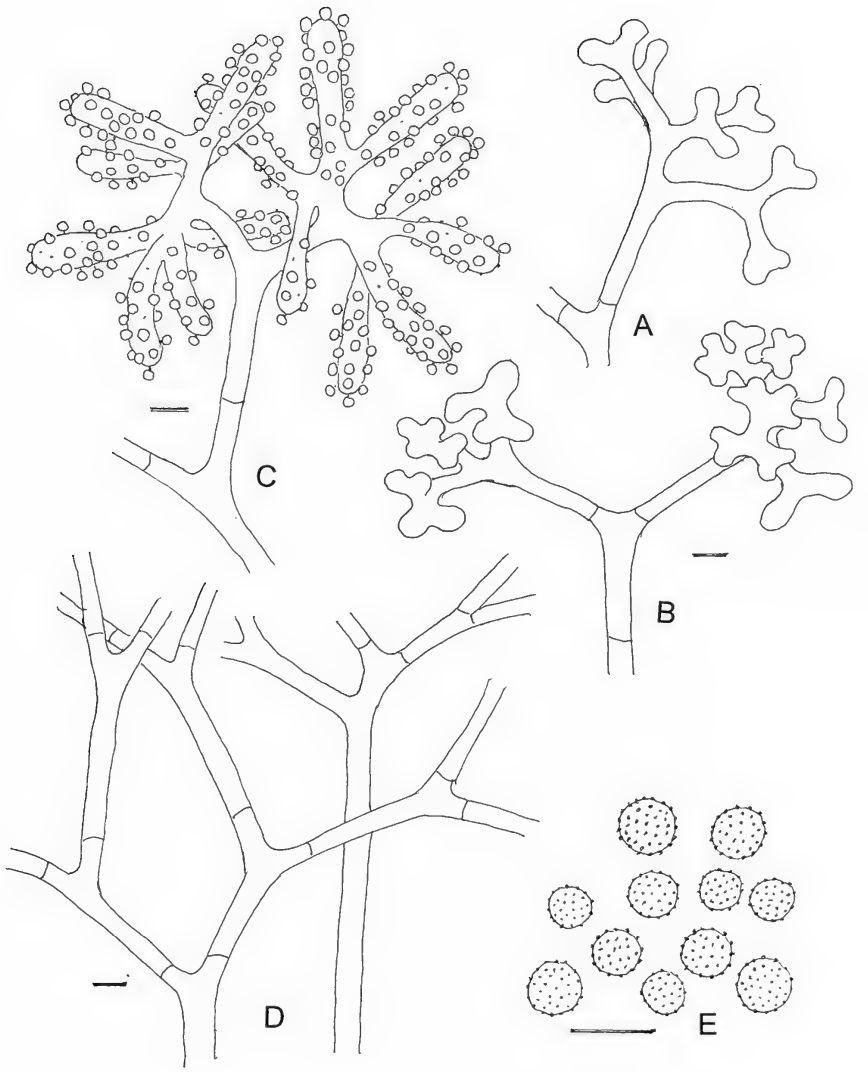

Fig. 5. *Chromelosporium canadense* (holotype, DAOM 71947). A. Young conidiogenous cells on terminal dichotomies. B. Primordia of the conidiogenous cells. C. Conidiogenous cells. D. Dichotomous branching of the conidiophore. E. Mature conidia. Scale bar = 10 μm.

Colonies in patches ≤15 mm wide and ≤3 mm thick, effuse, loose, first white, then tawny to cinnamon, composed of mixed hyphae and branched conidiophores bearing clusters of conidia.

Hyphae on substrate, thin, 4–7 µm, evanescent.

Conidiophores mononematous, erect, single, tawny, stipes 100–300 × 6–10 µm, then branching in regular and repeated dichotomies of different lengths, branches divergent at 20–60° angle, the basal ones 160–200 µm long, the distal ones shorter (16–48 µm), 10–12(–18) µm wide, the stipe and each branch septate at its base, the last dichotomy bearing two clusters of 4–8 primordial conidiogenous cells.

Conidiogenus cells formed as two groups of quadri-furcate terminal primordia that elongate up to 30–45 × 12–22 µm, slightly clavate, becoming conidiogenous over their entire length.

Conidia holoblastic, borne simultaneously on denticles, one-celled, globose, (3.5–)4–5(–7) µm, tawny, verrucose with 10–18 warts in median view, seceding rhexolytically.

Habitat: on rotten wood and bark, branches, mosses, plant stems and herbs, in gardens and forests.

Distribution: Canada and USA.

Comments—Growth on PDA was obtained from young conidiophore hyphae taken after 3 days at 4°C from fresh material of four collections (MUCL 1689 T, 1690, 1691, 1692) but not from conidia. Mycelium in culture is effuse, ochre, forming dense dispersed patches; hyphae are regular, septate, branching at an angle of c. 45°, or very irregular, closely septate in swollen cells. In two cases, small clusters of dark brown cells were produced, suggesting primordia of apothecia.

Herbarium specimens often lack the conidiogenous cells which are collapsed or absent; their presence is inferred from the branching pattern and the conidial characters.

Specimens examined:

Type & authentic material: **USA: Oregon:** (**2**) *Rhinotrichum carneum* Ell. & Ev., on bark, Oregon, 1885, W.S. Carpenter n° 125 [scr. Ellis] (NY).) [DAOM 84659, MUCL 2694]. (holotype) **New York:** (**3**) *Rhinotrichum carneum* Ell. & Everh., on rotten wood, Macoun [scr. Ellis]. Ellis Collection (NY) [DAOM 84677, MUCL 2839]. (**4**) *Rhinotrichum carneum* Ell. & Everh. [no substrate, no date], Macoun [scr. J.B. Ellis]. Ellis Collection (NY) [MUCL 2840].

Other specimens: **CANADA, Ontario** (**5**) *Hyphelia terrestris*, on *Tilia*, Nashville, York Co., Oct. 25 1953, coll. det. R.F. Cain (TRT 30178) [DAOM 84684, MUCL 2858]. (**6**) *Hyphelia terrestris*, on hardwood, Nashville, Nov. 14 1954, coll.

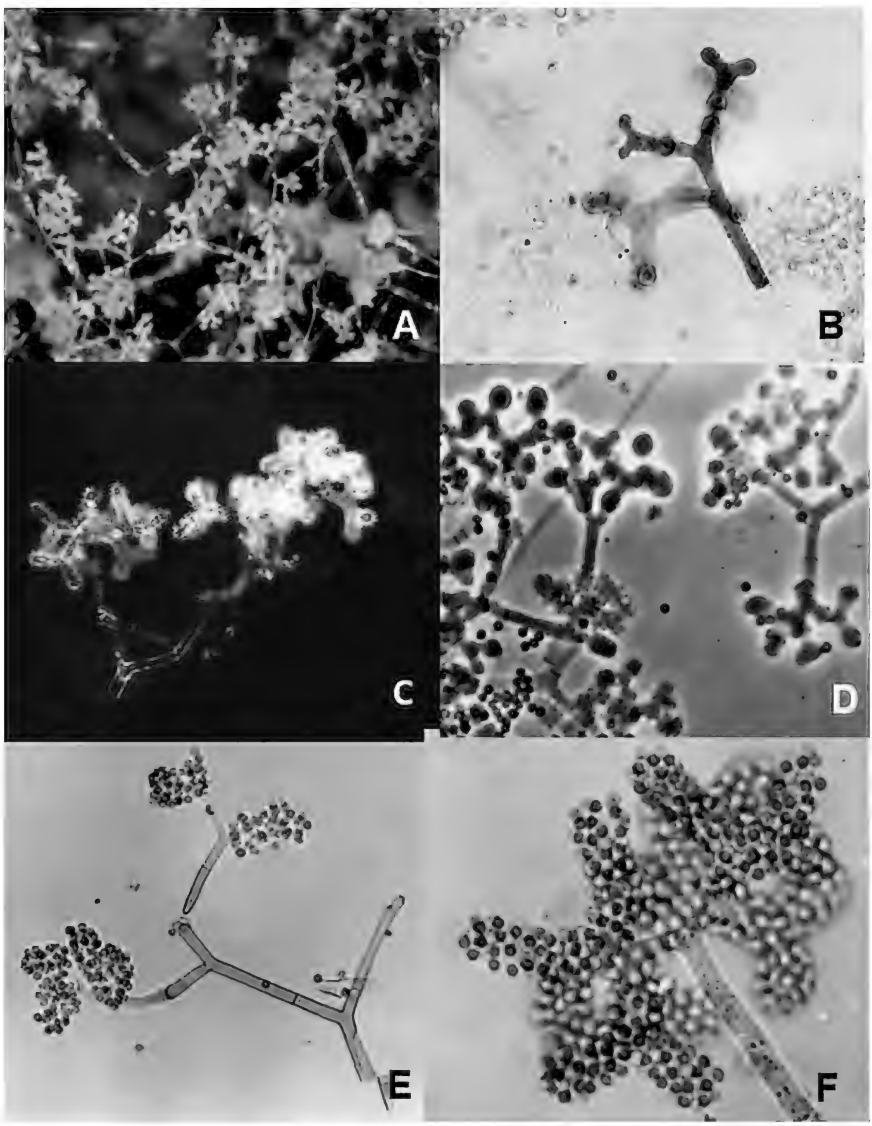

Fig. 6. *Chromelosporium canadense* (holotype, DAOM 71947). A. Conidiophores at low magnification. B-D. Young stages of conidiogenous cells. C. Half and fully mature conidioma. E. Wide angle of the dichotomies. F. Terminal bunch of mature conidiogenous cells.

det. R.F. Cain (TRT 31311) [DAOM 84683, MUCL 2857]. (7) *Hyphelia terrestris*, on *Populus* Nashville, Oct. 22 1955, coll. det. R.F. Cain (TRT 31699, BPI) [MUCL 2419]. (8) *Hyphelia terrestris*, on decayed log, New Durham, Brant Co., Oct. 14 1956 (TRT

32870) [DAOM 84688, MUCL 2862]. (**9**) *Botrytis fulva* Link, on dead wood, Oct. 20 1896, Macoun [Scr. J.B. Ellis]. Herb. Ellis (BPI) [DAOM 83886, MUCL 2415]. (**10**) *Botrytis* (*Phymatotrichum*) *fulva* Link, on decayed wood, N. Bathurst street Toronto, Nov. 17 1934, H.S. Jackson, det. D.H. Linder, Herb. R.F. Cain 8207 (TRT 8065, FH, DAOM 81409) [MUCL 2889)] (**11**) *Botrytis (Phymatotrichum) fulva* Link, on rotten wood, S. Aurora, Oct. 23 1932, H.S. Jackson, det. D.H. Linder. Herb. R.F. Cain 8210 (TRT 8060, DAOM 81410) [MUCL 2890]. (**12**) *Ostracoderma,* on soil of lawn, North Toronto, June 17 1960, S.J. Hughes. (DAOM 83325) [MUCL 2255]. (**13**) *Chromelosporium*, on bark and plant decay, South March, April 10 1962, G.L. Hennebert (MUCL 3067). **Québec** (**14**) *Ostracoderma* on bark, Gatineau Park, Nov. 25 1960, S.J.Hughes. (DAOM 71946) [MUCL 1688]. (**15**) *Ostracoderma,* on wood, Gatineau Park, Nov. 25 1960, S.J.Hughes. (DAOM 71948) [MUCL 1690]. (**16**) *Ostracoderma,* on wood, Gatineau Park, Nov. 2(5, 1960, S.J.Hughes. (DAOM 71949) [MUCL 1691]. (**17**) *Ostracoderma,* on wood, Gatineau Park, Nov. 25, 1960, S.J.Hughes. (DAOM 71950) [MUCL 1692]. (**18**) *Ostracoderma*, on wood and bark, Gatineau Park, Oct. 31, 1960, S.J.Hughes. (DAOM 71399) [MUCL 1689]. (**19**) *Ostracoderma*, on stem of *Veratrum viride*, Yoho Valley, Aug. 8 1960, S.J. Hughes. (DAOM71326) [MUCL 1513]. (**20**) *Chromelosporium,* on *Betula papyrifera* rotten wood, Gatineau Park, Oct. 31 1960, G.L.Hennebert. (DAOM 71545, MUCL 1590). (**21**) *Chromelosporium,* on rotten wood, Gatineau Park, Oct. 7 1960, G.L.Hennebert. (DAOM 71419, MUCL 1505). **Manitoba** (**22**) *Botrytis* near *phymatotricha* Sacc. prov. cfr. *lateritia*, C.E. Fairman suggests *Botrytis carnea* Schum, on old board, Winnipeg, Sept. 16 1928, G.R. Bisby 4140 [scr. Dearness]. Herb. Dearness 6732 (DAOM 84789) [MUCL 3070]. **USA, Ohio** (**23**) Mycobiota of Ohio. *Botrytis isabellina* Preuss, on rotten wood, The 7 Caves, Highland Co., coll. det. D.H. Linder 2667, Sept. 6 1933, Herb. W. Bridge Cooke (NY, FH, DAOM 84679) [MUCL 2847]. **Iowa** (**24**) *Hyphelia laxa* Schw., on decorticated conifer wood. (IA) [DAOM 83317, MUCL 2260]. **South Carolina**: (**25**) *Botrytis epigaea* Link var. on clay surface after rain, Sept. 1883, H.W.R. [scr. H.W. Ravenel]. Herb. U.W. Ravenel, (K-M, DAOM 83332) [MUCL 2281]. **Massachusetts** (**26**) *Botrytis*? *Hyphelia spectabilis* Harz, on coniferous bark, York, Sept. 14 1891. Herb. Thaxter 137 (FH) [DAOM 84702, MUCL 2886]. (**27**) *Botrytis fulva* Link, on hardwood branches on the ground, Beaver Brook reserve, Belmont, Oct. 3 1937, coll. G.D. Darker 64-90, det. D.H. Linder. (FH) [DAOM 84703, MUCL 2888]. **New York** (**28**) *Ostracoderma canadense*, on stem of fern in a pond, Forest Home, Ithaca, May 1 1962, G.L. Hennebert. (MUCL 3120). **New Jersey** (**29**) *Botrytis epigaea* Pers., on old pine boards partly buried in the ground, Newfield, N.J., Sept. 1881, Ellis. Ellis North American Fungi 827 (WIS, CUP, K-M, B, G, L) [MUCL 1804].

Chromelosporium arenosum Hennebert, Persoonia 7: 196, 1973. Fig. 7

Type: Flora Venezuelae 2474, on rotten wood of *Espeletia*, 3550 m, alt., Mucudaji, Sanide Santo Domingo, Estada Mesida, July 22 1958, coll. R.W.G. Dennis. (K-M) (**Holotype:** MUCL 2298 [DAOM 83359]; **isotype** IMI 75582 [MUCL 5889]).

Etymology: from the Latin *arenosus* or sand-like, suggesting the spreading granulose aspect of the sporulating conidiophores of ochraceous color seen under low magnification.

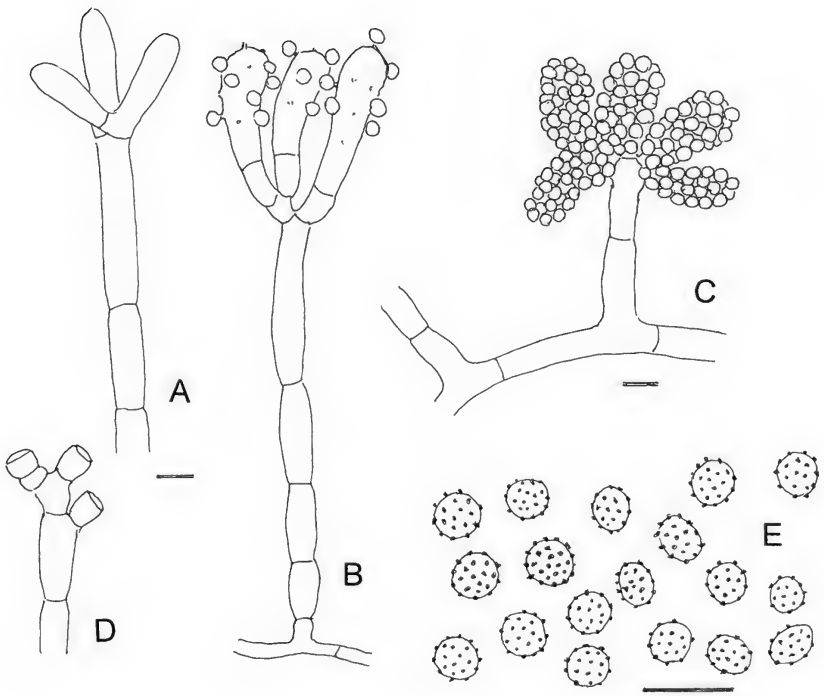

Fig. 7. *Chromelosporium arenosum* (holotype, DAOM 83359). A. Young conidiophore branching. B. Erect conidiophore with conidiogenous cells. C. Mature conidiophore with conidia borne on aerial hypha. D. Conidiophore after secession of conidiogenous cells. E. Conidia. Scale bar = 10 μm.

Colonies superficial, sparse, delicate, cobweb-like white mycelium, becoming arenose ochraceous with mature conidiophores appearing like minute, scattered grain.

Hyphae repent, hyaline, single, 7–9 μm wide, regular, straight, branched, thin-walled, smooth, septate with cells 7–12 μm long, sometimes regenerating after collapse from septum by internal narrow hyphal proliferations 3–4 μm wide.

Conidiophores arising singly, mononematous, as erect branches of the repent hyphae, short, 70–100 μm high, 8–9 μm wide, stipes with the basal cell bulbous 11–13 × 6–8 μm,, constricted at septa, cells 15–50 μm, apically branching in dicho- or trichotomies forming 2–8 terminal cells 40–50 μm long, obtuse, clavate up to 13 μm wide and conidiogenous.

Conidia holoblastic, borne simultaneously on denticle 1 × 0.5 µm, one-celled, globose or ovoid, 3.5–5.5 × 3.5–6.5(–8.1) µm, mostly 4.5 µm wide, 0.5–1 µm thick-walled, the outer wall hyaline, the inner wall subhyaline to ochraceous and coarsely verrucose, with 6–10 globoid or elongate warts in median view, seceding rhexolytically.

Habitat: on rotten bark of *Espeletia* sp. in the Tropics.

Distribution: Venezuela.

Chromelosporium macrospermum Hennebert, Persoonia 7: 197, 1973. Fig. 8

Type: living and dried cultures from *Peziza* sp. ind. on sterilized soil in greenhouse, Heverlee, Belgium, 2.04.1960, leg. G.L. Hennebert (**Holotype:** MUCL 1116; **isotype** DAOM 67492 = dried culture of MUCL 1116).

Colonies on malt agar substrate thin, white to rust-color when sporulating, comprising interwoven and prostrate hyphae, hyaline to subhyaline, producing laterally large spherical cells, 40–50 µm diam, thin-walled.

Conidiophores mononematous, erect, pale to rust-colored, septate, stipes 400–600 × 15–18 µm, apically bi- or quadri-furcate, branches 20 µm long, few septate, the distal ones erect, radiate, cylindrical to clavate 130–160 × 20–25 µm, conidiogenous.

Conidia holoblastic, borne on a denticle, more or less simultaneous, globose, rust-colored, 15–23 (av. 19.7) µm, thin-walled, finely punctate, seceding rhexolytically.

Habitat: sterilized greenhouse or nature soil

Distribution: Europe: Belgium, Oceania: Australia.

Comments – This unique strain MUCL 1116 was found in one ascospore germinating from several apothecia then identified as *Peziza ostracoderma,* collected in a tropical greenhouse of the Faculty of Agronomy UCL in Heverlee, Belgium in 1960. Korf differentiated the size and ornamentation of the ascopores preserved in lactic acid mounted slides of the original *Peziza* apothecia (Hennebert & Korf 1975) but in the absence of the single original apothecium, he could not circumscribe the species. The same species has been isolated from soil in South Australia by Dr. G.C. Hansford at the University of Adelaide in 1953 and preserved as living culture IMI 54710 in the culture collection at Egham and as dried culture in K-M–IMI 54710 under the name *Phymatotrichum* tax. sp. 7. The culture is *C. macrospermum* mixed with conidia of *P. ostracoderma* and unidentified hyaline chlamydospores.

The species might be a mutant *Peziza ostracoderma* or a distinct undetermined species of *Peziza*.

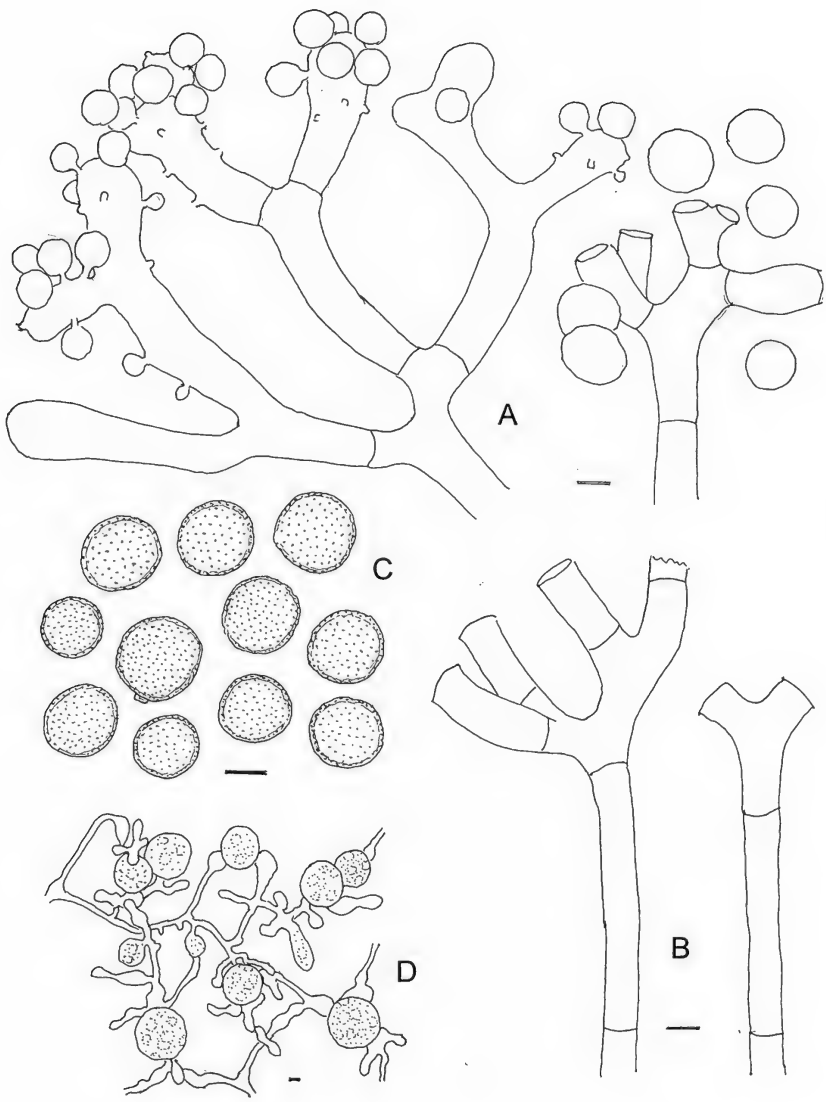

Fig. 8. *Chromelosporium macrospermum* (ex holotype, MUCL 1116). A-B. Conidiophores at diverse stages. B. Punctate conidia. D. Swollen cells in culture. Scale bar = 10 µm.

Species of *Pezizaceae* with known sexual morphs and similar mononematous conidiophores and conidiogenesis

Peziza ostracoderma Korf, Mycologia 52: 650, 1961 ["1960"]. FIG. 9

≡ *Plicaria fulva* R. Schneid., Zentralbl. Bakteriol. Parasitenk. Infektionskrankh. Hyg., 2. Abt., 108: 147, 1954 [non *Peziza fulva* Pers. 1822, nom. sanct.].

= *Dematium ollare* Pers., Syn. Meth. Fung. 2: 697, 1801.

≡ *Botrytis fulva* Link, Spec. Plant., ed. 4, 6(1): 58, 1824, nom. illeg. [superfluous].

≡ *Sporotrichum fulvum* Fr., Syst. Mycol. 3: 418, 1832, nom. sanct. [not "(Link) Fr."; non Link 1809].

≡ *Trichosporum fulvum* (Fr.) Fr., Summa Veg. Scand. 2: 492, 1849.

≡ *Polyactis fulva* (Fr.) Bonord., Handb. Mykol.: 115, 1851 [not "(Link) Bonord."].

≡ *Chromelosporium fulvum* (Fr.) McGinty, Hennebert & Korf, Mycologia 67: 216, 1975 [not "(Link) McGinty et al."].

≡ *Chromelosporium ollare* (Pers.) Hennebert, Persoonia 7: 197, 1973.

= *Haplaria nitens* Delacr., Bull. Soc. Mycol. France 6: 140, 1890.

= *Botrytis luteobrunnea* Krzemien. & Badura, Acta Soc. Bot. Poloniae 23: 727, 1955 (1954).

= *Mycotypha dichotoma* F.A. Wolf, J. Elisha Mitchell Sci. Soc. 71: 217, 1955.

≡ *Ostracoderma dichotomum* (F.A. Wolf) Matsush., Icon. Microfung. Matsush. Lect.: 103, 1975.

TYPES: ***Plicaria fulva* R. Schneid.**, on damp sterilized soil in greenhouse, Berlin-Dahlem, Germany, 25.3.1953 (**Holotype: B;** authentic material in CUP R.P.K. 4114).

***Dematium ollare* Pers.**: dried culture from living culture of *Plicaria fulva* R. Schneider, on damp sterilized soil, greenhouse, Berlin-Dahlem, Germany, March 1953, received in Febr. 1954 from R. Schneider (**Neotype:** MUCL 1112, designated in Hennebert 1973; **isoneotypes:** dried culture DAOM 81809, living cultures CBS 382.54, CCRC 36608, IMI 059206).

COLONIES velvety to floccose, white when young, yellow to fulvous or cinnamon when sporulating, rapidly growing.

HYPHAE mostly prostrate, interwoven, branched, septate, anastomosing, hyaline to pale fulvous.

CONIDIOPHORES mononematous, solitary or gregarious, erect and divergent, arising from prostrate or aerial hyphae; stipe 130–600 × 8–17 µm, hyaline to fulvous, sparsely septate, attenuate at the base, apically 1–3 times dichotomously branched, dichotomies short, 10–25 × 5–13 µm, septate at the base, the terminal ones clavate, slightly inflated ≤15 µm, conidiogenous.

CONIDIOGENUS CELLS producing conidia along their length, collapsing after release and seceding away at basal septa, leaving the stipe or the lowest dichotomies as a stump.

CONIDIA holoblastic, borne simultaneously on a denticle 3 × 1 µm, densely and irregularly spaced, globose or napiform, 5–9(–14) µm (mean = 7.5 µm), fulvous, wall thin, finely punctate, seceding rhexolytically. Conidia

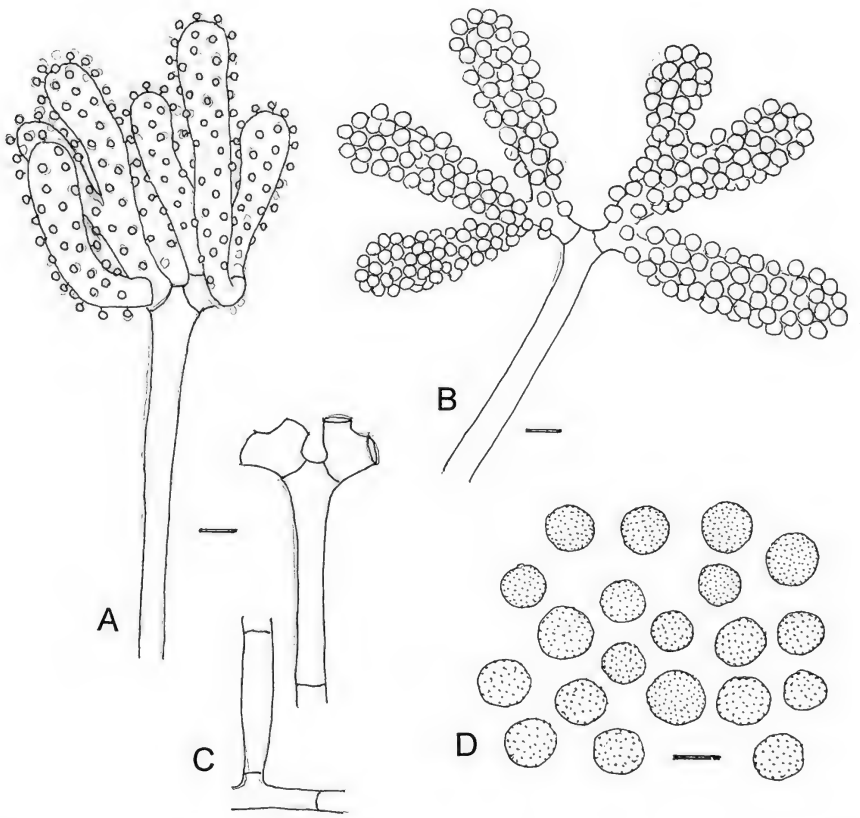

Fig. 9. *Peziza ostracoderma* (neotype MUCL 1112). A-C. Conidiophores at diverse stages. D. Punctate conidia. Scale bar = 10 µm. The magnification of the line-drawings of conidia in figures 8 and 9 is half that in other figures.

germinate and colonies sporulate profusely on malt agar and diverse organic poor culture media.

Habitat: On sterilized soil in greenhouses, on mushroom beds, on cardboard, paper pots, paper, and old textiles in moist chambers, on humid plaster walls in houses, on moist hay, on dung of rodents, and on forest soil.

Distribution: Europe, North America, Asia.

Comments—Korf (1961) knew the species in both its asexual and sexual morphs in the greenhouses at Cornell when he received the type and a living culture of *Plicaria fulva*. The species has elliptical ascospores like most *Peziza* species. Seeing the sanctioned name *Peziza fulva* Pers., he created the new

name *Peziza ostracoderma*, referring to Hughes' opinion that the conidial morph could be referred to *Ostracoderma*. Hennebert (1960) reported and described the same fungus observed in a tropical greenhouse of the Faculty of Agronomy UCL in Heverlee, Belgium, in 1960.

Hennebert (1973) segregated *Chromelosporium* from *Ostracoderma,* which Hughes (1958) had considered synonymous. He named the conidial morph of *P. ostracoderma, Chromelosporium ollare,*, with *Dematium ollare* as basionym, which he neotypified with GLH 1112 (MUCL 1112) in the absence of an original authentic specimen from Persoon.

Hennebert & Korf (1975) cited *Dematium ollar*e as the earliest post-Linnean name for the conidial morph common in greenhouses. Link (1824) transferred the species to *Botrytis* as *Botrytis fulva,* an illegitimate name with *Dematium ollare* cited as its unique synonym. Fries (1932a) excluded *D. ollare* from his genus *Dematium*, treating it, after Link, as a synonym of *B. fulva,* both of which he transferred into *Sporotrichum* as S. *fulvum,* a sanctioned name.

Searching for the earliest possible name to combine in *Peziza* in accordance with the 2017 Shenzhen ICN, it is apparent that the earliest name of the fungus, *Dematium ollare*, cannot be used, because of *Peziza ollaris* Fr. (Fries 1822), a different species, the name of which is sanctioned over *Peziza ollaris* Schaeff. (Fungorum in Bavaria et Palatinatu circa Ratisbonam Icones 4:126, 1774) and *Peziza ollaris* Pers. (Mycol. Eur. 1: 299, 1822), which might be the present species.

Despite what is posted on Index Fungorum, the names *Alytosporium fulvum* (Link) Link and *Nodulisporium fulvum* S. Hughes, being based on *Sporotrichum fulvu*m Link, are synonymous with neither *Dematium ollare* nor *Botrytis fulva.*

Haplaria nitens, added here as a synonym, was collected on sterilized oak barks from a tannery used as mulch in a warm glasshouse of the Laboratoire de Pathologie végétale de l'Institut national Agronomique in Paris. Its conidia are minutely verrucose.

The variable conidiophore length and variable number and length of the dichotomies of the species might explain its frequent confusion with *Chromelosporium ochraceum*. The primary diagnostic differences reside in conidial size and ornamentation and the ecological habitat.

In an attempt to clarify the relationship with *Chromelosporium macrospermum,* the variability of conidial size in monoascospore strains of *Peziza ostracoderma* was studied on different culture media (malt-agar,

potato-dextrose-agar, carrot-agar, and jiffy-agar, a mixture of soil and peat). Although conidial size varied between 5–9 µm and one strain produced conidia ≤12 µm and a different strain produced conidia ≤14 µm, none reached 15–23 µm, the conidial size reported for *C. macrospermum* of.

Type specimen examined. **EUROPE: France:** (2). *Haplaria nitens* Del. n. sp. Paris (scr. Delacroix). (holotype, PC) [isotypes, DAOM 59453, MUCL2700]

Other specimens examined See Hennebert & Korf (1975).

Plicaria trachycarpa (Curr.) Boud.,

Hist. Class. Discom. Eur. 50, 1907, **var. *trachycarpa*** Figs 10, 11

≡ *Peziza trachycarpa* Curr., Trans. Linn. Soc. London 24: 493, 1864.

≡ *Discina trachycarpa* (Curr.) P. Karst., Acta Soc. Fauna Fl. Fenn. 2(6): 113, 1885.

≡ *Detonia trachycarpa* (Curr.) Sacc., Syll. Fung. 8: 105, 1889.

≡ *Curreyella trachycarpa* (Curr.) Massee, Brit. Fung.-Fl. 4: 401, 1895.

≡ *Lamprospora trachycarpa* (Curr.) Seaver, Mycologia 6: 19, 1914.

≡ *Plicariella trachycarpa* (Curr.) Velen., Monogr. Discomyc. Bohem.: 342, 1934.

≡ *Galactinia trachycarpa* (Curr.) Le Gal, Bull. Trimestriel Soc. Mycol. France 78: 212, 1962.

= *Rhinotrichum trachycarpum* F.A. Wolf, J. Elisha Mitchell Sci. Soc. 74: 166, 1958.

= *Chromelosporium trachycarpum* Hennebert, Persoonia 7: 197, 1973.

Types: ***Peziza trachycarpa* Curr.**, supra solum deustum, Ascot com. Surrey, Nov. 1862 (**Holotype:** K(M) 29980).

***Chromelosporium trachycarpum* Hennebert**, asexual morph of *Peziza trachycarpa,* on burned area, Camp II, Allegany State Park, USA: June 11, 1961, coll. & isol. G.L. Hennebert, det. R.P. Korf (**Holotype,** DAOM 83324; **isotype,** MUCL 2197).

Colonies fast growing on malt agar medium, pellicular, white to pale ochraceous.

Hyphae thin, hyaline, interwoven and prostrate.

Conidiophores mononematous, erect, very short, 50–100 × 8–10 µm, repeatedly dichotomously or assymetrically branched, irregularly septate, branches cylindrical, short, 20–35 × 8–10 µm, widely divaricate, becoming totally conidiogenous, forming compact globose conidial heads.

Conidia holoblastic, borne simultaneously on denticle, yellow-ocher, one-celled, globose or shortly ovate, 5–7 × 5–9 µm, wall finely verrucose, with 10–15 warts in median view, seceding rhexolytically.

Habitat: in moist burned area in forests.

Distribution: Northern Hemisphere.

Comments—The drawings were made while I was in Canada from an ascospore culture of the species collected in Ontario (MUCL 2339) identified

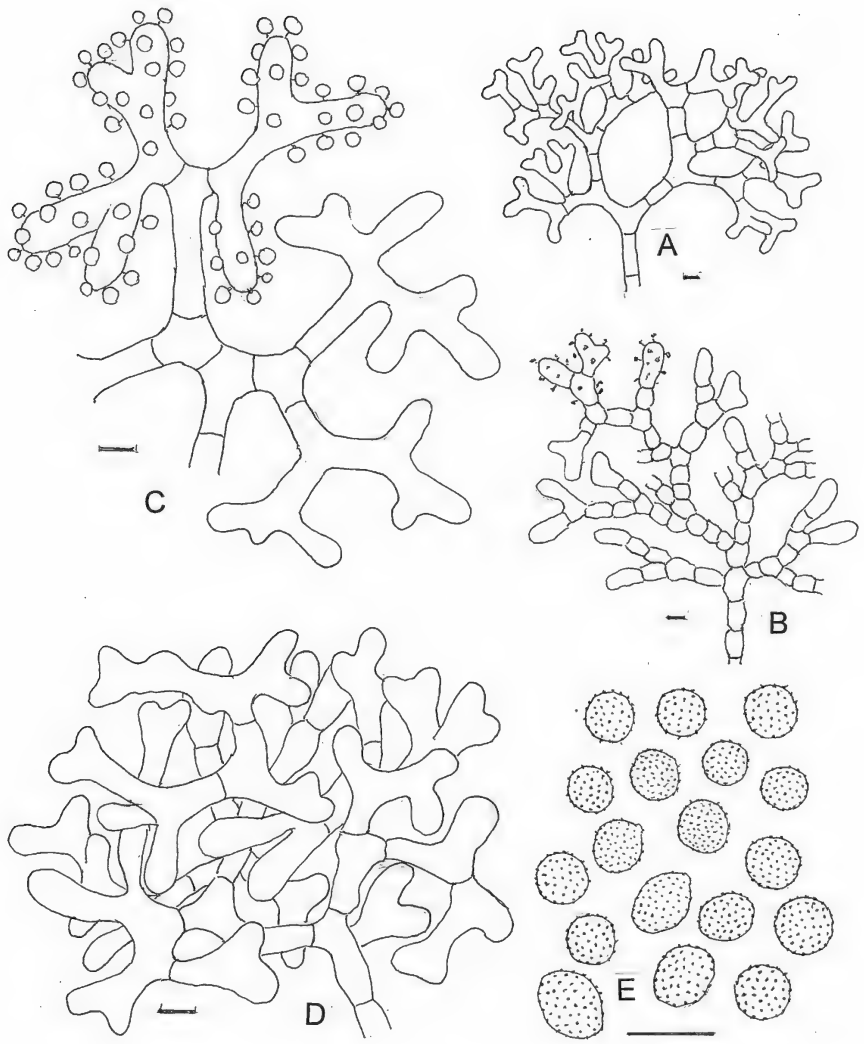

Fig. 10. *Plicaria trachycarpa*. A-B. Branched conidiophore. C. Terminal conidiogenous cells. D. Much septate conidiophore on porcupine dung (MUCL 2933). E. Punctate conidia. (A-C and E on malt agar MUCL 2339). Scale bar = 10 µm.

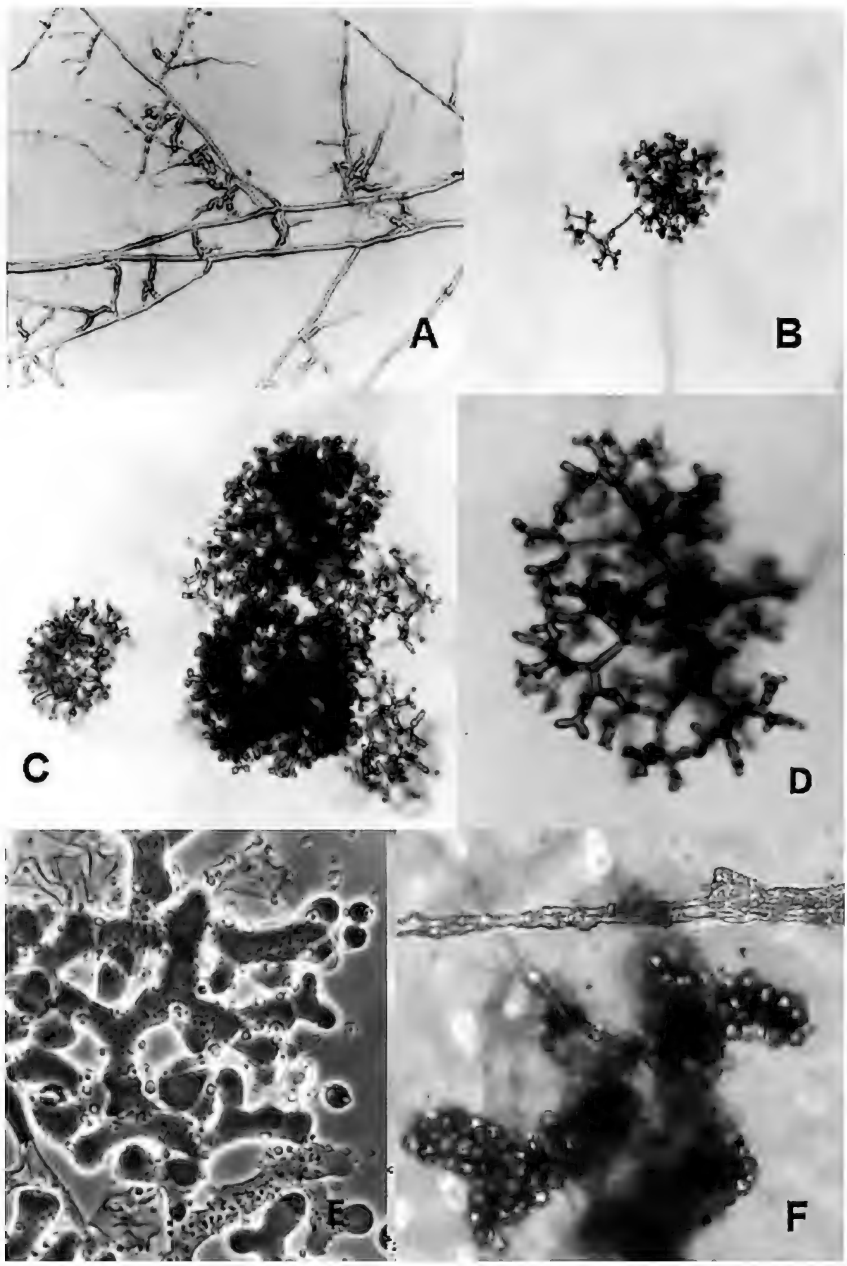

Fig. 11. *Peziza trachycarpa*. A. Initiation of conidiophores. B-D. Globular branched conidiophores showing the dichotomies. E. Initiation of conidia. (A-E, MUCL 2339). F. Mature conidiogenous cells (DAOMC 199631 = MUCL 57201 on malt-agar).

as identical to DAOM 83324 but not after examination of K(M) 29980. A living strain labelled as *Plicaria trachyspora* var. *muricata* (= *Plicaria carbonaria* (Fuckel) Fuckel 1870) was recently received as DAOMC 199631 that, on 2 % malt agar, developed similar clusters of dichotomous branches close to the substrate and bearing mature conidia.

Specimens examined;

NORTH AMERICA: USA, New York (2) *Peziza trachycarpa* Currey, on burned area, State Line Run, near Onoville, Cattarangu Co., June 10 1961, coll. Kumi T Korf, det. R.P. Korf (MUCL 2198). (3) *Chromelosporium*, on porcupine dung (in moist chamber) from Stoddard Brook Road, Allegany State Park, June 11 1961, coll. & isol. G.L. Hennebert. (DAOM 89363, MUCL 2933). **CANADA, Ontario** (4) *Peziza trachycarpa* Currey, on moist clay soil in mixed woods, Bell's Corners, July 31 1961, coll. & isol. G.L. Hennebert 2339. (DAOM 83382, MUCL 2339). (5) *Plicaria trachycarpa* (Currey) Boudier var. *muricata* Grelet on burned litter in burned mixed forest, Renfrew Co., Ont., Sept. 2 1979, K.N. Egger 0281, dried and culture (CCF 6918, DAOMC 199631, MUCL 57201).

EUROPE: UK (6) *Peziza trachycarpa* (Curr.) Boud., conidia on cardboard flower pot., Evesham, Worcs., March 25 1955, col. R.E. Taylor, det. R.W.G. Dennis. Dried culture IMI 59800) [MUCL 3514].

Chromelosporiopsis

Chromelosporium-like species with synnematous conidiomata resemble mononematous *Chromelosporium* species in having the same conidiogenesis and ornamented globose conidia but differ in the conidiophore fasciculation and asymmetric sparsely septate dichotomous branching (a branching pattern described here as coralloid). This concerns two asexual species named by Hennebert (1973)—*Chromelosporium carneum* and *C. coerulescens*—both with sexual morph unknown. For these species, the new generic name *Chromelosporiopsis* is proposed. Similar asexual morphs have also been described in some *Pachyphlodes* species of the *Pezizaceae*.

Chromelosporiopsis Hennebert, **gen. nov.**

MB 835622

Differs from *Chromelosporium* by its synnematous conidiophores and irregular successive bifurcate branching.

Type species: *Chromelosporiopsis carnea* (Schumach.) Hennebert

Etymology: *Chromelosporium* + *-opsis*, similar to.

Ascomycota, *Pezizaceae*, sexual morph unknown.

Hyphae septate, branched and anastomosed, fasciculating in synnemata, forming defined or effuse cushions, diversely colored (white, ochre, yellow, rose, flesh red, blue, or violet).

Conidiophores synnematous, laterally and/or apically branching in irregular successive dichotomies called here a coralloid pattern, sparsely septate, apically slightly inflated.

Conidiogenus cells subterminal and terminal branches forming synchronously along their lengths holoblastic conidia, each on a denticle; secession rhexolytic.

Conidia one-celled, globose or subglobose, with thick ornamented (finely to coarsely verrucose) wall, colored in mass. seceding rhexolytically.

Habitat: on organic soil.

Chromelosporiopsis carnea (Schumach.) Hennebert, **comb. nov.** FIGS 12–14, 15A

MB 835623

≡ *Botrytis carnea* Schumach., Enum. Pl. 2: 238, 1803, nom. sanct.

= *Isaria carnea* Pers., Ann. Bot. (Usteri) 15: 13, 1795; Syn. Meth. Fung. 2: 689, 1801.

≡ *Chromelosporium carneum* (Pers.) Hennebert, Persoonia 7: 196, 1973.

= *Polyactis carnea* Ehrenb., Sylv. Mycol. Berol. 25, 1818.

≡ *Mucor carneus* (Ehrenb.) Link, Spec. Plant., ed. 4, 6(1): 88, 1824, nom. illeg., non Schaeff. 1774

≡ *Botrytis carnea* (Ehrenb.) Spreng., Syst. Veg., ed. 16, 4(1): 551, 1827, nom. illeg., non Schumach. 1803.

≡ *Sporodinia carnea* (Ehrenb.) Wallr., Fl. Crypt. Germ. 2: 317, 1833.

≡ *Ostracoderma carneum* (Ehrenb.) S. Hughes, Canad. J. Bot. 36: 792, 1958.

= *Botrytis rosea* Link, Mag. Ges. Naturf. Freunde Berlin 7: 36, 1815, nom. illeg., non DC. 1805.

= *Botrytis linkii* Duby, Bot. Gall, 2: 919, 1830 [as "*linckii*"].

≡ *Ostracoderma linkii* (Duby) S. Hughes, Canad. J. Bot. 36; 792, 1958.

= *Campsotrichum splendidum* Schwein.,Trans. Amer. Philos. Soc., n.s. 4: 283, 1832.

≡ *Botrytis splendida* (Schwein.) Sacc., Syll. Fung. 4: 123, 1886.

= *Rhinotrichum opuntia* Berk. & Broome, Ann. Mag. Nat. Hist., ser. 2, 13: 462, 1854.

= *Botrytis carnea* f. *foliicola* Roum., Fungi. Sel. Gall. Exs., Cent. 14: no. 1367, 1881.

Types—***Polyactis carnea*** **Ehrbg.**[scr. Ehrenberg] on leaves of *Fagus sylvatica,* Herb. Schwaeningrichen (STR) (**MBT 392408, here designated as lectotype** [DAOM 83897, MUCL 2440]). *Polyactis carnea* Ehrbg. [scr. Ehrenberg] on leaves of *Fagus sylvatica,* Herb. Nees (STR) (**Isolectotypes:** DAOM 83896, [MUCL 2439]; Herb. Persoon (L 910-262-747) [MUCL 2464]).

Chromelosporium carneum **(Pers.) Hennebert** on leaves of *Quercus pedunculata* and *Fagus sylvatica*, Forest de Soignes, Tervueren, Belgium, Aug. 18 1960, leg. G.L. Hennebert (**Epitype:** MUCL 1208, designated as "neotype" by Hennebert, 1973; **isoepitype:** DAOM 74697).

Colonies superficial, fast growing and evanescent, in scattered tufts of hyphae, 3–15 mm wide, 15–2 mm high, comprising ≤15 erect divergent synnemata

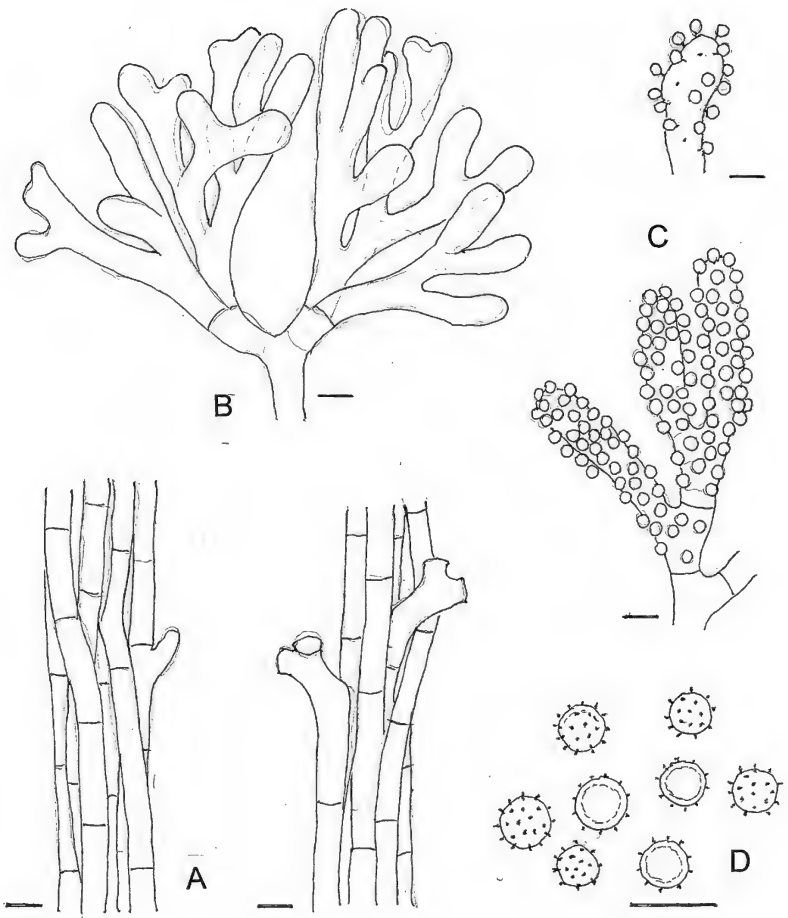

Fig. 12. *Chromelosporiopsis carnea*. A Synnemata (MUCL 1806) .B. Coralloid branching of the conidiophores. C. Conidiogenous cells. D. Verrucose conidia (B-D, isolectotype MUCL 2464). Scale bar = 10 µm.

bearing conidia in their upper half, white when young, flesh-colored when mature on the field, ochraceous salmon when dried.

Hyphae repent, sparse, delicate, hyaline to ochraceous, single or aggregated, (9–)12–15(–23) µm wide, thin-walled, smooth, septate, branched, often anastomosing, with short cells.

Synnemata arising from the basal mat of hyphae, erect columnar, ≤1.5 mm high, 50 µm wide, composed of ≤30 growing conidiophores.

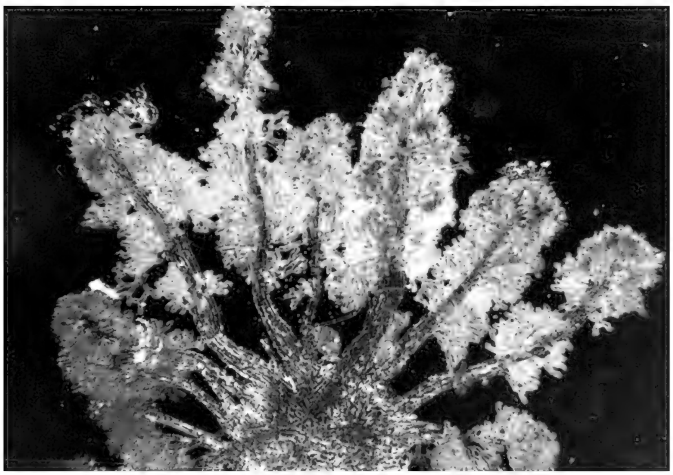

Fig. 13. *Chromelosporiopsis carnea*, cluster of synnemata with isarioid conidial head (MUCL 6278).

Conidiophores stipes contiguous, 11–17 µm wide, anastomosing, septate at 50–80-µm intervals, thin-walled, smooth, slightly bent outwards laterally and apically in the upper half of the synnemata and branching in a coralloid pattern of asymmetric dichotomies, one branch of each dichotomy developing first and sometimes becoming septate at the base,

Conidiogenous head formed of 4–9 last successive bifurcations, the branches being 15–50 × 6–10 µm, apically obtuse, slightly clavate, 7–12 µm wide and often bent, all branches conidiogenous, forming an isarioid head.

Conidia holoblastic, borne simultaneously on denticle 1 × 0.5 µm, 5–11 µm distant from each other, one-celled, globose, occasionally subglobose, 4.8–6.6(–7.8) µm (most 5.4–6 µm), walls 1–1.5 µm thick, the inner wall ochraceous, the outer wall cyanophilic and coarsely verrucose, bearing 8–12 rounded warts in median view, seceding rhexolytically.

Habitat: on dead leaves of *Fagus sylvatica* and *Quercus pedunculata*, also on other leaves, bark, mosses, and organic debris on soil, in forests, from August to October.

Distribution: Europe: Austria, Belgium, France, Denmark, Germany, Great Britain, Netherlands, Sweden.

Comments—The earliest description and illustration of *Chromelosporiopsis carnea* is by Persoon (1795) under the name *Isaria carnea*, with conidiophores sticking together ("conferta"), at first white, then flesh-colored ("primo albida dein carnea") and evanescent ("evanescens"), illustrated in Persoon (1796: Observ. Mycol. 1, tab. 2 figs 6-7) by fine color paintings (Fig. 15A). The

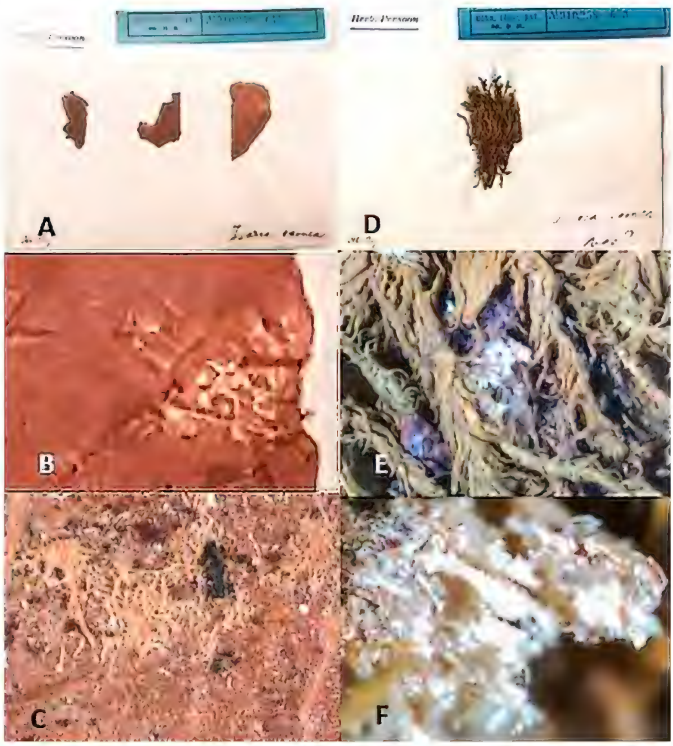

Fig. 14. Authentic specimens of *Isaria carnea* Pers. A-C. *Isaria carnea* [written by Persoon]. A. *Isaria carnea* [written by Persoon]. A. Three pieces of *Fagus* leaves. B. Enlarged immature colony. C. Enlarged collapsed synnemata. D-F. *Isaria carnea var.*? [written by Persoon]. D. Patch of moss. E-F. Enlargements of a small patch of the fungus showing young white isarioid synnemata. (Photos J. Nuytinck and Dr M. Scherrenberg, Naturalis Museum, Leiden).

authentic specimens of *Isaria carnea* in Persoon's herbarium, signed by Persoon but with no date, are here documented by the excellent photographs made by Dr Nuytinck and M. Scherrenberg in Leiden (Fig. 14). Schweinitz (1832) showed that *Isaria carnea* has fascicles of hyphae (synnemata) diverging from a shared base, pale brown, apically floccose and bearing abundant fleshy-red spores ('sporulis carneo-rubris') spread along the hyphae. *Isaria carnea* was treated by Fries (1832a,b) as a synonym of *Dactylium macrosporum* Fr., nom. sanct. [≡ *Botrytis macrospora* Ditmar, nom. illeg.], but it is really a different fungus, and and its epithet is therefore potentially available for use.

However the epithet is not truly available, as Fries (1832a) accepted and sanctioned the species under the name *Botrytis carnea* based on Schumacher's description and his examination of Ehrenberg's specimens of *Polyactis carnea*.

Botrytis carnea is described as "cespitosa, stipitibus brevissimis, dichotomis, ramosissimis, albidis, sporulis congestis spicatis carneis; in lingo putrido *Betulae albae*, Septembr," while *Polyactis carnea* shows "floccis suberectis dichotomis capitatis albis, sporidiolis primum albis, dein carneis, globosis. In foliis pinorum. Pluvio freq. In fossis. Septembr." As neither Schumacher nor Ehrenberg cited Persoon, they are two distinct names for the same species, not obligate synonyms. As the name *Botrytis carnea* is sanctioned it must serve as the basionym for the species as understood here.

No original material of *Botrytis carnea* is available, but three good specimens collected by Ehrenberg and preserved in STR and L under the name *Polyactis carnea* contain synnemata, bifurcate branching hyphae, and verrucose conidia.

Campsotrichum splendidum Schwein. n° 2695 is described with synnemata ("coalitis floccis"), furcate branches emerging along the upper half, and bearing reddish orange verrucose conidia ("pulcherrime decorate"). This is a possible synonym of *C. carneum*.

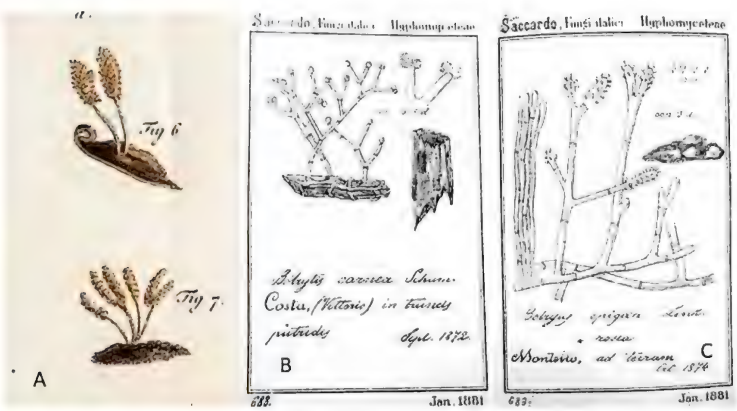

Fig. 15. A. Persoon's illustration of *Isaria carnea* in Observ. Mycol. 1: tab. II, fig. 6-7, 1796. B-C. Saccardo Fungi Autografice Delineati (1881), fig. 688: *Botrytis carnea* Schum. and fig. 689: *Botrytis epigaea* var. *rosea* Sacc.

Saccardo (1881) illustrated *Botrytis carnea* (Fig. 15B) as mononematous and dichotomous with verruculose, rose-isabella conidia without indication of synnemata. However, he drew synnemata and asymmetric bifurcations in *Botrytis epigaea* var. *rosea*, another synnematous *Chromelosporium*-like taxon (Fig. 15C). Saccardo (1886) also described *B. carnea* var. *quercina* with smaller conidia, 4.5–5 µm, asperulate and rose-colored. Both of these varieties show characters of *C. coerulescens*.

Specimens examined.

Authentic specimens: **EUROPE:** (**5**), *Isaria carnea* [scr. Persoon] on leaves of *Fagus* leaves, Herb. Persoon (L 910-258-645). (**6**) *Isaria carnea var?* [scr. Persoon] on a patch of moss. Herb. Persoon (L 910-258-663) [the specimen bears immature smooth conidia]. **Germany,** (**7**) *Botrytis rosea* Link, on small pieces of leaves of *Fagus sylvatica*, Rostock. Herb. Link (B) [DAOM 51764, MUCL2451]. **UK,** (**8**) *Rhinotrichum decolorans* Forden, on cupules of *Fagus sylvatica*, Forden 312. Herb. M.C. Cooke 1885 (K-M) [MUCL 3495].

Other specimens: **EUROPE, UK** (**9**) *Phymatotrichum*, on bare soil and dead leaves, Ashridge, Herts., England, July 17 1955, D.A. Reid (K-M) [MUCL 2304]. (**10**) *Phymatotrichum sp.* on soil and vegetable debris, Windsor Great Park, Windsor, Oct. 3 1962, D.A. Reid & R. McNabb (K-M) [MUCL 3133, MUCL 3494]. (**11**) *Botrytis carnea*, on *Epilobium hirsutum*, Witham Park, Oxfordshire, Sept. 17 1949, M.B. & J.P.Ellis (K-M–IMI60211) [MUCL 3517]. (**12**) *Botrytis* aff. *splendida* Schw., on mossy *Salix* bark, Wheatfen Broad, Nrf., M.B. Ellis (K-M–IMI 26983) [MUCL 3568]. (**13**) *Phymatotrichum* sp. on petioles of *Petasites ovatis*, Masham, Yorks., Oct. 13 1947, S.J. Hughes (K-M–IMI 19231b) [MUCL 3572]. (**14**) *Phymatotrichum*, bark of *Quercus*, Kinclaven, Perthshire, Sept. 1953, M.B. Ellis (K-M–IMI63926) [MUCL 3505]. (**15** *Phymatotrichum* sp., on bark and earth in a deep hole, Ashridge, Hertford. July 17 1956, D.A. Reid, det. M.B. Ellis (K-M–IMI 60584) [MUCL 3520]. (**16**) *Phymatotrichum* sp., on rotten wood, Ruislip Woods, Oct.14 1955, C. Booth, det. A.H.S.. Brown. Herb.(K-M–IMI 61356) [MUCL 3523]. (**17**) *Phymatotrichum,* on moss on beach, Burnham, Beeches, Oct. 20 1956, A.H.S. Brown. (K-M–IMI 69690) [MUCL 3541]. (**18**) *Phymatotrichum.,* oak and hazel leaf litter, Park Wood, Ruislip, Oct. 15 1949, P.K.C. Austwick (K-M–IMI 37957) [MUCL 3559]. (**19**) *Chromelosporium carneum*, decaying *Quercus* wood, Pett near Hastings, Sussex, Aug. 29 1965, P.C. Holland, det. W. Gams (MUCL 7929). (**20**) *Chromelosporium carneum*, on stem debris, Ashdown Forest, Forest Row, Sussex, Oct. 1 1967, D. Mitchell & P.C. Holland (MUCL 11290). **Netherlands** (**21**) *Botrytis fulva* Link ex Fr. on herbaceous stems under *Fagus sylvatica*. Bussum, Oct. 24 1929, W.J. Lütjeharms (L) [DAOM 83937, MUCL 2515]. **Austria** (**22**) *Botrytis carnea* Schum. ad folia et cortices putridos, sylvis umbrosis, autumno, L. Fuckel.. Fuckel Fungi. Rhen. Exs. 146 (G, K-M, GRO, NY) [DAOM 74694, MUCL 1808]. **Denmark** (**23**) *Phymatotrichum*, on leaves, wood and cupules of *Fagus sylvatica*, Gris Skov, Sealand, Oct. 2 1955, S.J. Hughes (DAOM 51702). (**24**) *Phymatotrichum*, on wood chips of *Fagus sylvatica*, Gris Skov, Sealand, Oct. 2 1955, S.J. Hughes (DAOM 51690). **Poland**:(**25**) *Botrytis carnea* Schum., in trunco *Juniperi*, Eichler, in herb. Bresadola 184 (S) [DAOM 83948, MUCL 2532]. (**26**) *Chromelosporium carneum*, on rotten wood, Biatowiesga forest, Pologne, Sept.5 1966, W. Gams (MUCL 9298). (**27**) *Chromelosporium carneum*, on rotten wood, Augustow Reserve, Starozym, Pologne, Sept.3 1966, W. Gams (MUCL 9299). **France** (**28**) *Botrytis fulva* Link écorce de *Quercus* et *Corylus,* Lyon, Sept. 1879, J. Therry. C. Roumeguère Fungi Gall. Exs. (BR) [DAOM74695, MUCL 1809]. (**29**) *Botrytis carnea.* feuilles de chêne [*Quercus*] pourrissantes, Tassin, Lyon. Sept. 1880, Cryptogames du Lyonnais, J.J. Therry. 5092 (PAD) [MUCL 1870]. (**30**) *Phymatotrichum,* on mossy bark of *Quercus*, Forêt de Jupilles, France, Sept.15 1952, E.M. Wakefield, det. M.B. Ellis (K-M–IMI 50643) [MUCL 3598]. **Belgium** (**31**) *Botrytis rosea* Link, in foliis

Quercus robur, coll. Libert (BR) [DAOM 74693, MUCL 1806]. (**32**) *Chromelosporium carneum*, on dead leaves of *Fagus sylvatica* and *Quercus pedunculata* in woods, Parc d'Arenberg, Hevelee, Sept. 1 1960, G.L. Hennebert (MUCL 1218, DAOM 74698). (**33**) *Chromelosporium carneum*, on twig of *Fagus sylvatica*, Parc d'Arenberg, Hevelee, Août 21 1964, G.L. Hennebert (MUCL 6278). (**34**) *Chromelosporium carneum*, on bare humid soil under *Fagus sylvatica*, in Lauzelle forest, Louvain-la-Neuve, Oct. 2017, G.L. Hennebert (MUCL 56651).

Chromelosporiopsis coerulescens (Bonord.) Hennebert, **comb. nov.** FIGS 16–18

MB 835625

≡ *Polyactis coerulescens* Bonord., in Fresenius, Beitr. Mykol. 1: 14, 1850.

≡ *Botrytis coerulescens* (Bonord.) Sacc., Syll. Fung. 4: 132, 1886.

≡ *Chromelosporium coerulescens* (Bonord.) Hennebert, Persoonia 7:197, 1873.

= *Hyphelia purpureospadicea* Fuckel, Jahrb. Nassauischen. Vereins Naturk. 23–24: 363, 1870.

≡ *Botrytis purpureospadicea* (Fuckel) Sacc., Syll. Fung. 4: 121, 1886.

= *Botrytis epigaea* subsp. *rosea* Sacc., Michelia 2(8): 544, 1882.

≡ *Botrytis epigaea* var. *rosea* (Sacc.) Sacc., Syll. Fung. 4: 136, 1886.

= *Botrytis carnea* var. *quercina* Sacc. Syll. Fung. 4: 119, 1886.

TYPES: *C.[Chromelosporium] rhodianthinum* n.sp.-[prov.name] on rotting leaves and humic debris of *Acer saccharum*, *Betula lutea* and *Tsuga canadensis* in mixed woods, Bell's Corners, Ontario, Canada, 18.07.1961, leg. G.L. Hennebert (**Neotype:** DAOM 83371, designated in Hennebert 1973; **Isoneotype:** MUCL 2323).

COLONIES in tufts, appearing and rapidly evanescent, with ≤20 synnemata arising from a basal mat of hyphae, at first white to bluish, sky-blue turning to rose-lilac, finally vinaceous ocher at maturity in fresh conditions and vinaceous brown when dried.

HYPHAE superficial, sparse, delicate, hyaline, thin-walled, smooth, forming mats 1–5 mm wide, 1 mm high, hyphae interwoven 11–29 µm wide, septate into 32–46 µm long cells.

SYNNEMATA erect, divergent, composed of ≤25 hyphae, septate and anastomosing, the central hyphae growing first ≤800 µm high, the outermost ones shorter, each hyphae serving as a conidiophore stipe.

CONIDIOPHORES stipes hyaline, 6–9 µm wide, septate, with 36–54 µm long cells, thin-walled, smooth, all branching at the top, in successive, asymmetric dichotomies in a coralloid pattern, forming a globose or somewhat conical conidial head.

CONIDIOGENOUS HEAD comprising all branches of the conidiophore except the first ones, 15–75 (mostly 30–60 µm) µm long, 9–12 µm wide, the distal cells clavate ≤15 µm wide, obtuse, forming conidia almost simultaneously.

CONIDIA holoblastic, on denticle 1 × 0.5 µm, 4–9 µm spaced, one celled, globose, 4.2–6.6 µm, most 5.4 µm, walls 1 µm thick, subhyaline, the outer

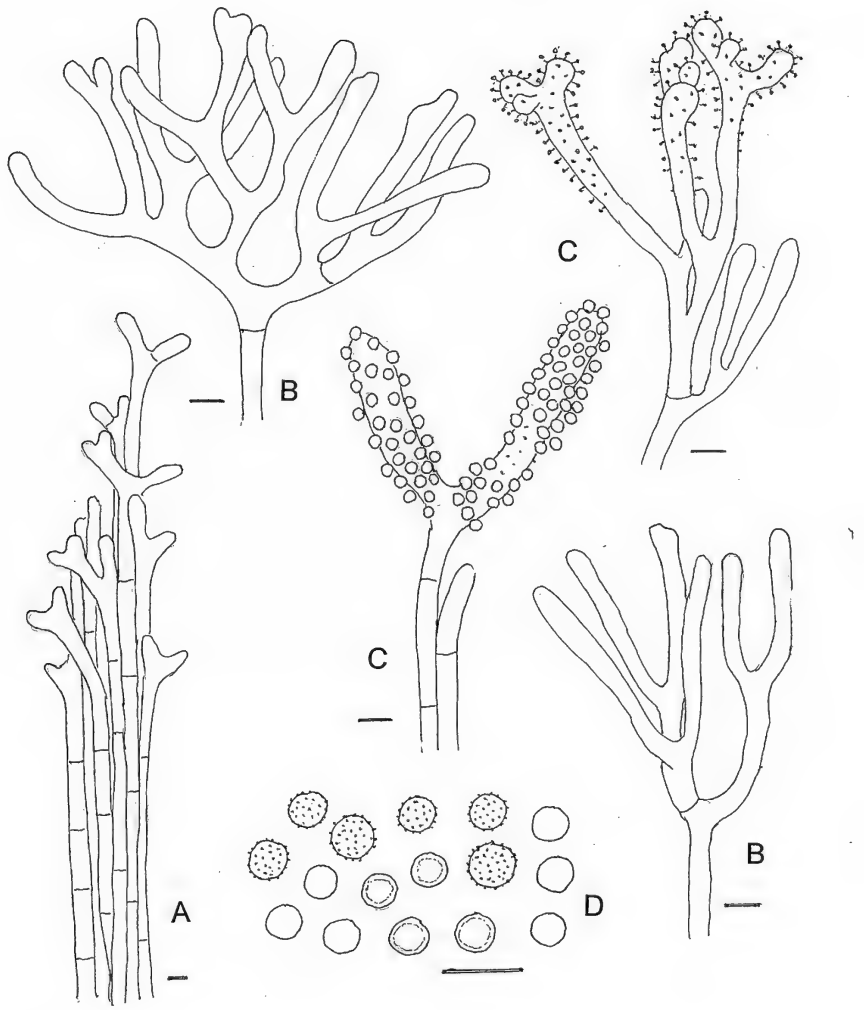

Fig. 16. *Chromelosporiopsis coerulescens* (neotype DAOM 83371).
A. Synnemata B. Asymmetric dichotomies or coralloid branching. C. Conidiation.
D. Finely verrucose conidia. Scale bar = 10 µm.

wall finely punctate ('minutissime asperulis', Saccardo 1886 p. 132), with 12–18 small warts in median view, seceding rhexolytically.

Habitat: on mosses, rotten wood debris, tree leaves, conifer needles in humid forest.

Distribution: North America: Canada, USA, Europe: France, Belgium.

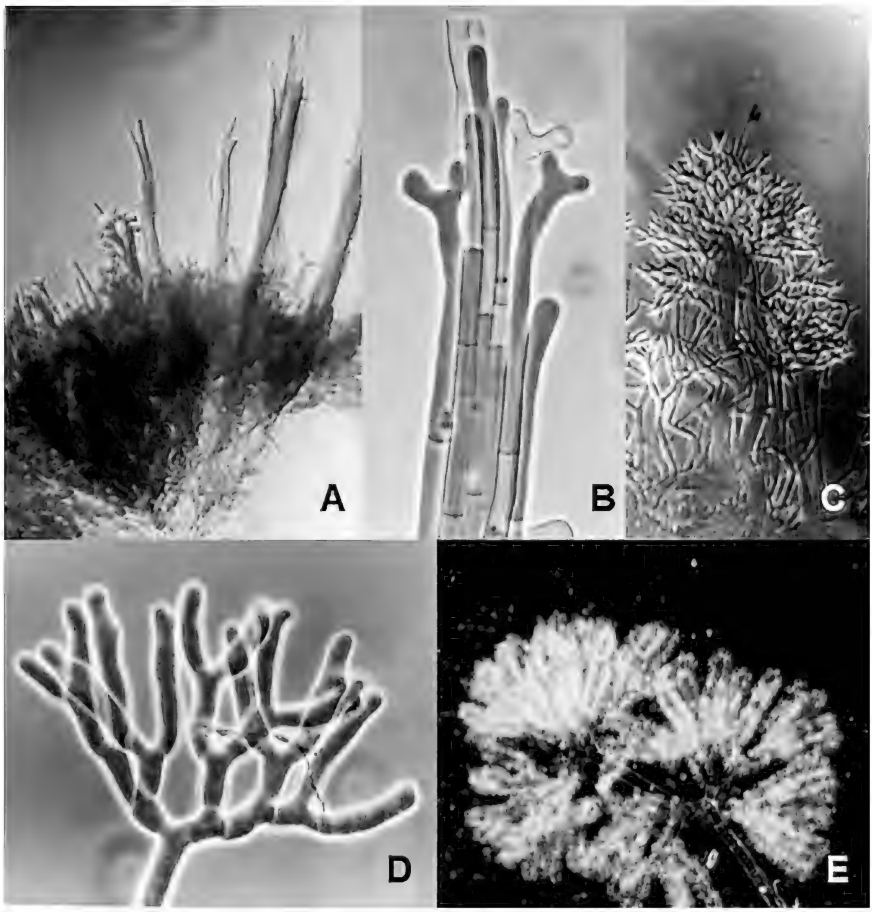

Fig. 17. *Chromelosporiopsis coerulescens* (neotype DAOM 83371). A. Fungus mat with emerging young synnemata. B. Top of growing synnema. C. Head of branches. D. Asymmetric dichotomous branching. E. Conidial head.

Comments—Fresenius (1852: 74), who received a specimen and a description of the species from Bonorden, noticed that "die sporen sind rund, feinwarzig, nicht glatt wie Bonorden angibt". Indeed Bonorden (1851) described the spores as "glatt". Fresenius also illustrated the conidiogenesis of the species.

Chromelosporiopsis coerulescens differs mainly from *C. carnea* by the colour when young, the synnematal development, the narrower conidiophore hyphae, and the smaller, punctate conidia.

Bonorden (1851) described *Polyactis coerulescens* as "zuerst weisse, dann hellblau, zuletzt duch die Sporen bräunlich." The pale blue (hellblau) young

Fig. 18. *Chromelosporiopsis coerulescens.* A. Young blue cushions and white young synnemata. B. Rose-lilac nearly mature synnemata (specimens collected and photographed on rotting trunk on the ground, A. 14.06.2014, Sainte-Cécile-de-Masham, QC and B. 19.08.2018, Orleans, Ont, Canada, by Jonathan Mack, Mycoquebec.org).

stage of the colonies changes during maturation to rose-lilac-mauve and then vinaceous ochre-brown.

Fuckel (1870) described under *Hyphelia* the new species *H. purpureospadicea*. It is here a synonym of *C. coerulescens* after examination of the holotype.

Labbé (2015) describes the species as "remarquable par ses colorations bleu cristal puis rose-violet et ressemblant presque à des cristaux en forme d'aiguilles." The species is illustrated in its change of colour in the photographs by Jonathan Mack (Fig. 16) in J. Landry on mycoquebec.org and in photographs by Cornell Mycology (Hodge 2017).

Specimens examined

Type & authentic specimens **EUROPE: Germany** (2) *Hyphelia purpureospadicea* Fuckel, ad terram arenosam humidam in sylvis acerosis, raro, Autumno, circa Budenheim, Fuckel. Fungi Rhen. Exs. 2214 (G, holotype; BR, GRO, PAV, FH, isotypes) [DAOM 83395, MUCL 2396].

Other specimens. **NORTH AMERICA**: **Canada** (3) *Botrytis* ?, on humus, Morgan's woods, MacDonald College, near Montreal, Qué., Aug. 27 1941, R.F. Cain 12979 (DAOM 80136) [MUCL 2276]. **USA** (4) *Botrytis dichotoma*, on ground and rotting wood, Fall Creek, Ithaca, N.Y., June 18 1894, G.F. Atkinson. Herb. Atkinson 1153 (CUP) [DAOM 84693, MUCL 2876]. (5) *Botrytis terrestris*, on humic ground under conifers, Shelburne, New Hampshire, Sept. 1891, W.G. Farlow (FH) [DAOM 84699, MUCL 2883]. (6) *Botrytis terrestris*, on dead leaves, trunks, dungs, etc. in conifer woods, Shelburne, W.G. Farlow (FH) [DAOM 84700, MUCL 2884]. (7) *Botrytis epigaea*, on rotten wood and mosses, Hadley Lake, Machias, Sept. 21 1898, W.G.

Farlow 36 (FH) [DAOM 84698, MUCL 2882]. (**8**) *Botrytis epigaea* Link, on mosses, Magnolia, Mass. July, W.G. Farlow (FH, UPS, CUP) [DAOM 84912, MUCL2484]. (**9**) *Botrytis epigaea*, on *Hydnum*, Gerrish Island, Kittery Pt., Maine, R. Thaxter (FH) [MUCL 2892]. (**10**) *Botrytis (Polyactis) coerulescens* (Bon.) Sacc., on bare soil in forest, Lingle Valley, Mifflin Co., Pa., July 3 1938, L.O. Overholts 21176, D.H. Linder (FH) [MUCL 2891]. (**11**) *Hyphelia terrestris*, on decaying leaves, Ringwoods, N.Y., Sept. 6 1952, R.F. Cain (TRT 24143) [MUCL 2863]. (**12**) *Ostracoderma,* on *Quercus* rotten wood, Pennsylvania state, July 4 1957, E.A. Atwell n° 5779 (DAOM 59233). (**14**) *Ostracoderma*, on lawn, College Park, Maryland, Sept. 15 1961, A.M. Golden, C.R. Benjamin (BPI) [MUCL 2957].

EUROPE: **Belgium** (**15**) *Hyphomycete* n° 517, sur sol de jardin, Rue Berchmans, Bruxelles, June 21 1917, M. P. C. Beeli (BR). (**16**) *Botrytis violacea/lilacina* Schw, sur argile, Forêt de Soignes, Sept. 23 1933, Bommer (BR).

Species of *Pezizaceae* with known sexual morphs and similar synnematous conidiophores and conidiogenesis

A similar *Chromelosporiopsis* asexual morphology was found in specimens collected by Korf (1994) and identified by Healy & al. (2015) as *Pachyphlodes pfisteri* Tocci & al. (*Pezizaceae*), and briefly described by Hennebert (2017).

In October 2017, some samples of synnematous *Chromelosporiopsis* conidial mats were collected on bare soil in forests around Louvain-la-Neuve in Belgium. The DNA sequencing in MUCL identified three samples as *Pachyphlodes nemoralis* Hobart & al., and one sample as *P. citrina* (Berk. & Broome) Doweld. Their conidial morphs are clearly distinguished by the synnematal structure and hyphal width, the hyphal length and width of the conidiophore coralloid branches, and particularly by the conidial size and the conidial wall ornamentation (Hennebert & Decock 2020).

Pachyphlodes nemoralis has globose conidia, 4–6.5 µm wide, with 0.5 µm thick walls covered with ≤0.3 µm high tuberculate warts (12–14 in median view).

Pachyphlodes citrina has globose conidia, 4.5–7 µm wide, with 0.5 µm thick walls covered with ≤0.8 µm high baculate warts (12–14 in median view).

3. Doubtful and excluded taxa

Most synnematous *Chromemosporium*-like herbarium specimens examined were labeled as *Botrytis epigaea*, *Polyactis epigaea*, *Hyphelia terrestris,* or *Phymatotrichum*, among others. These names are considered below.

Botrytis epigaea Link, Spec. Plant., ed. 4, 6(1): 52, 1824.

≡ *Polyactis epigaea* (Link) Bonord., Handb. Mykol.: 115, 1851.

≡ *Phymatotrichum epigaeum* (Link) Vasyag., in Shvartsman & al., Flora Sporov.. Rast. Kazakhst. 8: 263, 1973.

No authentic material of *Botrytis epigaea* or *Polyactis epigaea* was retrieved from relevant herbaria. Link (1824) originally described *Botrytis epigaea* as: "*Botrytis epigaea,* thallo effuso, floccis sporodiferis subramosis brevibus, sporidiis globosis minutis. Habitat in terra humida sylvarum Germaniae. Lecta Berolini im Thiergarten (Lk.) (v.v.)." This nine-word description being applicable to a number of fungi, the name is doubtful in absence of authentic specimen. Fries (1832a,b) did not accept the species in *Sporotrichum* because he considered it as only mycelium.

Bonorden (1851) interpreted Link's species under *Polyactis epigaea* in his Fig. 161 as a fungus showing bifurcate conidiophores covered with conidia, adding that the colour of the mature mass of spores is gray or greyish yellow ("grauen oder graugelben Pulver"). This might suggest a *Chromelosporium*-like species, but no authentic material is extant.

The earliest specimen received as *Botrytis epigaea* was collected by Fuckel in 1861. This synnematous *Chromelosporium*-like fungus with punctate conidia does not possess enough for identification.

Saccardo (1886) considered *Botrytis epigaea* similar to *Hyphelia terrestris* sensu auct.

Phymatotrichum Bonord., Handb. Mykol.: 116 (1851).

Bonorden, who defined his genus with conidiophores branched tree or shrub-like, not umbellate, bearing on their distal swollen branches pecidellate conidia ("gestielte Sporen"), described three species: *P. gemellum, P. pyramidale*, and *P. laneum* (Fig. 19).

While *P. pyramidale* Bonord. was transferred to *Botryosporium*, Saccardo interpreted *P. laneum* as *Botrytis laneus* (Bonord.) Sacc. and *P. gemellum* as *B. gemella* (Bonord.) Sacc.; Hennebert (1973), who interpreted *P. gemellum* as *Botrytis cinerea* Pers., deduced that *Phymatotrichum* was a synonym of *Botrytis*.

But none of the researchers considered the pedicellate conidia ("gestielte Sporen") mentioned by Bonorden as a diagnostic generic criterion, which is neither a character of *Botrytis* nor of *Phymatotrichopsis* (Fig. 20). *Phymatotrichum gemellum* is described with some fasciculate superficial hyphae ("verbundenen hypha") bearing short tufts ending in unequal

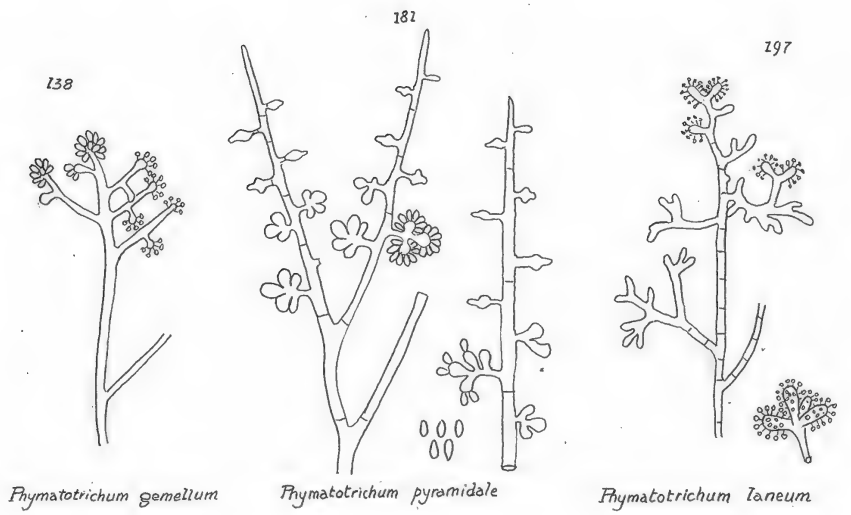

Fig. 19. Copy of the Bonorden's illustrations of the species of *Phymatotrichum*.

bifurcate apical swollen fertile cells ("Endäste erweitern zich zu zwei ungleich grossen Basidien"), characters that possibly suggest species of *Chromelosporiopsis*. But Bonorden commented that the species possessed characters of both *Polyactis* and *Botrytis*, but absent any authentic material, the genus remains doubtful.

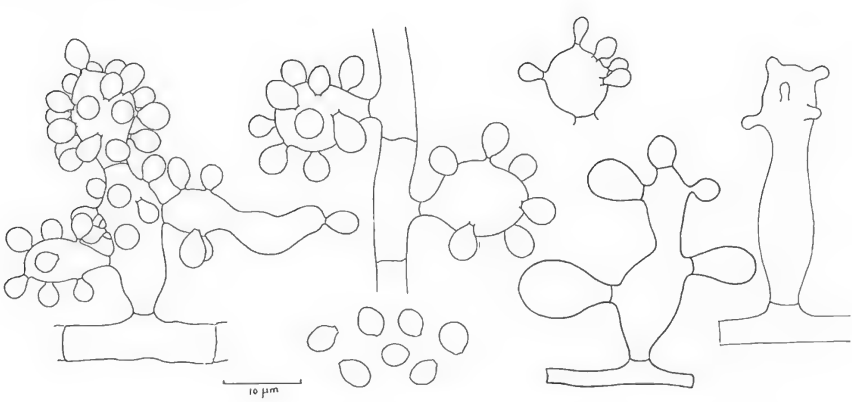

Fig. 20. Conidiogenesis of *Phymatotrichopsis omnivora* (Shear) Hennebert from dried sporemat on soil in cotton field, Paris, Texas, Sept. 18, 1915, BMD, received from Missouri Bot. Garden June 1916 (FH) [MUCL 2868] (Marek et al. 2009).

Phymatotrichum silvicola Taubenh. & G.M. Watkins, Amer. J. Bot. 24: 390, 1937 [as "*silvicolum*"].

SPECIMENS EXAMINED: (**1**) *Phymatotrichum silvicolum* Taubenhaus and Watkins, on post oak wood on sandy soil near Bryan, Brazos Co, Texas, November 1936 (NY) [DAOM 84656, MUCL 2690]. (**2**) *Phymatotrichum silvicolum* Taubenhaus and Watkins, on post oak wood on sandy soil near Bryan, Brazos Co, Texas, Sept.8, 1937 (BPI) [DAOM 84657, MUCL 2691].

Two authentic specimens of *Phymatotrichum silvicola* were received. The fungus is described from Texas on sandy soil in forest, as "hyphal mats more or less circular, 0.5–4 cm wide, white becoming greyish yellow, with synnemata of conidiophores arising from them, with bifurcate branching, the apical branches clavate, 5–8 µm wide and covered with subglobose one-celled conidia 2–4.6 µm wide on denticles". The specimens are ambiguous, containing globose (3.5–5.2 µm) punctate conidia mixed with napiform to globose (6.5–8.7 µm) verrucose conidia.

Rhinotrichum thwaitesii Berk. & Broome, Ann. Mag. Nat. Hist., ser. 2, 7: 177, 1851.

SPECIMEN: *Rhinotrichum thwaitesii* Berk. & Broome, Clifton, Berkeley. Herb. Berk. (K-M), holotype, with included watercolour plate by Berkeley [MUCL 2952].

The specimen contains conidiophores branched in dichotomies bearing globose conidia on denticules with very verrucose walls, but no synnemata have been seen. *Rhinotrichum thwaitesii* remains a doutful species in view of the discrepancies between the fungus and Berkeley's accompanying watercolour plate and the published diagnosis of the name with ovoid and larger spores.

Isaria thyrsoidea Penz. & Sacc., Malpighia 15: 251, 1902.

Isaria thyrsoidea, found on rotting leaves, insects and dung, in Tjibodas, Java, 1897, is described as white tufts of erect cylindrical synnemata 1.5 mm high, 40–50 µm wide, made of septate hyphae that emerge laterally and apically from the upper half of the synnemata as bi- and tri-furcated conidiogenous branches, 5.5–6 µm wide, sparsely septate and covered on denticle ("subsessile") with smooth globose conidia 3.5–4 µm wide (FIG. 21), The fungus is turns ash-grey at maturity. Are the conidia really smooth? Despite the colour of the

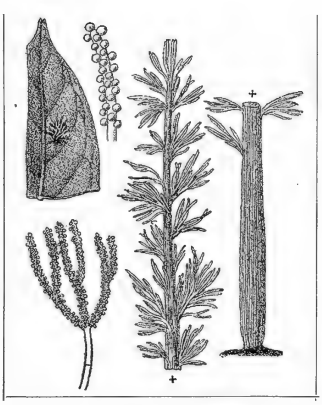

Fig. 21. *Isaria thyrsoidea* Penzig & Sacc., Malpighia 15: 251, 1902

fungus and the smooth conidia, the description suggests a *Chromelosporiopsis* species. The original material needs to be located and re-studied.

Chromelosporium terrestre (Fr.) M.B. Ellis, More Demat. Hyphom.: 154, 1976.

≡ *Hyphelia terrestris* Fr., Syst. Mycol. 3(1): 213, 1829, nom. sanct.

Ellis (1976) proposed the combination *Chromelosporium terrestre* for the basionym *H. terrestris* in order to correct the then illegitimate name *Chromelosporium tuberculatum* (Hennebert 1973). But *Hyphelia terrestris* is not a *Chromelosporium*-like species as shown below. The fungus described and illustrated by Ellis under that name is an unidentified species of *Chromelosporiopsis* and belongs to the set of collections considered hereunder.

Unidentified herbarium material with features of *Chromelosporiopsis*

Beside the presence of synnemata, a generic character, the specific microscopic characters of *Chromelosporiopsis* (observed when possible in the following material) are the hyphal widths (diameters), the branching patterns, the condial size, and the ornamentation of the conidial wall. The conidial wall ornamentation serves as an important criterion distinguishing between asexual morphs of *Pachyphlodes* species (Hennebert & Decock 2020) and is used here as a basis for a preliminary grouping of the specimens. But a good characterization of the wall ornamentation requires high quality equipment without which the present groups are based only on the number of warts visible on median view of the conidia and not on the wall morphologies. The specimens are classified according to the conidial wall surfaces ranging from punctate to coarsely warty. To be diagnostic, the number of median warts must be combined with wart morphology.

Specimens examined

Conidia punctate (≥18 warts, median view)

Authentic specimen **EUROPE:** (**3**) *Trichoderma laeve* ? Pers., frequens in sylvulis prope Parisios [scr. Pers.] Herb. Pers. (L 910.267.31) The fungus is immature and fragmentary. [DAOM 83899, MUCL 2461].

Other specimens. **EUROPE**: **Germany** (**4**) *Botrytis epigaea* Link, *Polyactis epigaea* Bon., Giesen, Oct. 22.1861, Herb. Fuckel in herb. Barbey-Boissier (G 005439) MUCL 2400)]. (**5**) *Botrytis epigaea* Link, ad terram Bavaria, Killermann. Herb. Bresadola (BPI) [DAOM 83891, MUCL 2428]. **Sweden** (**6**) *Hyphelia terrestris*, ad terram nudam, Stollsbachen, Uppsala, Aug. 7 1932, Seth Lundell. Flora Suecica (UPS) [DAOM 83921, MUCL 2494]. (**7**) *Hyphelia terrestris*, at the lower course of the rivulet Skytebäcken, Hälsungland, Färila parish, Skyte, Aug. 2 1956, J.A. Nannfeldt. Flora Suecica 14770 (UPS) [DAOM83905, MUCL 2770]. (**8**) *Hyphelia terrestris*, Zögenern Wald, b. Lündensitz, Aug. 1844 (PR 181907) [DAOM 84707, MUCL 2907]. **UK** (**9**)

Ostracoderma, on wet soil, Wotten under Edge, Gloucestershire, Aug. 1961, R.W.G. Dennis (K-M–IMI 89255) [DAOM 83951, MUCL 2541]. (**10**) *Botrytis splendida* Schw., on soil, Skircoat Green, Halifax, Yorkshire, Nov. 1955, R.Watling, det. M.B.Ellis (K(-M–IMI 61440) [MUCL 3524]. **Finland** (**11**) *Hyphelia terrestris* = *Botrytis epigaea* Link var. *rosea* Sacc. on soil, Mustiala, Aug. 25 1869, P.A. Karsten, W Nylanders, Herb. P.A. Karsten (H) [DAOM 83903, MUCL 2470]. (**12**) *Hyphelia terrestris*, supra terram, Mustiala, Aug. 1865, P.A.Karsten. Flora Fennica (UPS) [DAOM 83913, MUCL 2485]. **Netherlands** (**13**) *Ostracoderma* (? *Tomentella granulata* Bref.), op naakte bodem in loofbos lenig humensegrond, Ulvenhout, Ulvenhoutse bos, Sept. 29 1959, R.A. Maas Geesteranus 13006 (L) [DAOM 83935, MUCL 2513]. **Portugal** (**14**) *Botrytis carne*a Schum., ad terram, Portugal, Torrend 21, Herb. Bresadola (S) [DAOM 83946, MUCL 2528]. **Belgium** (**15**) *Hyphelia,* on bare soil, Forêt de Soignes, Tervueren, Aug. 16 1960, G.L. Hennebert (MUCL 1214) [DAOM 83904]. **Denmark** (**16**) *Ostracoderma*, ad terram in sylva faginea, Sjaelland, Sonnerup sogn., Ovdrup skov, Sept. 13 1952, Mykologisk Kongres, J.A. Nannfeldt. Flora Danica 12552 (UPS) [DAOM 83915, MUCL 2488].

NORTH AMERICA: CANADA (**17**) *Ostracoderma*, on garden soil, in gras, Ottawa, Oct.15 1960, V.J. (MUCL 1534). (**18**) *Botrytis epigaea*, on soil in barley field, Glen Williams, Halton Co., Ont., Sept. 9 1956, R.F. Cain (TRT 32547) [DAOM 84686, MUCL 2860]. **USA** (**19**) *Rhinotrichum thwaitesii* B. & Br., on ground in woods, south side of Fall Creek, Ithaca, June 25 1959, G.F. Atkinson, Herb Atkinson 22860 (CUP) [DAOM 84695, MUCL 2878]. (**20**) *Hyphelia terrestris* on ground, Nashville, York Co, Ont. Sept. 29 1956, R.F. Cain (TRT 32443) [DAOM 84685, MUCL 2859]. **USA** (**21**) *Botrytis isabellina* Pr., on bark of *Pinus sylvestris*, Jumby Lawn, Boston, Nov. 1906, H.C. Hawley (BM) [MUCL 2280]. (**22**) *Hyphelia terrestris*, on damp ground in woods, Bois Mallet, West La. May 23 1886, A.B. Langlois 412 (BPI) [DAOM 83892, MUCL 2429]. (**23**) *Hyphelia terrestris*, on sandy soil in field, Nashville, York. Co. Ont. Oct. 9 1955, R.F. Cain, Herb. RFC 31601 (WU) [MUCL 2408]. (**24**) *Botrytis epigaea* Link, on moist humus and soil, Altamonte, Florida, Aug. 1 1957, P.O. Schalbert, P.L. Lentz (BPI) [MUCL 2960].

AUSTRALASIA: Australia (**25**) *Phymatotrichum*, on dead leaves, twigs and woody fruits, Brisbane, Australia, June 1951, R.F. Langdon 817 (K-M–IMI 54841) [MUCL 3504]. **New Zealand** (**26**) *Botrytis terrestris*, snow white patch on soil, New Zealand, W. Odenso. Herb. J.B. Ellis (NY) [DAOM 84680, MUCL 2851].

Conidia verrucose (12–16 warts, median view)

EUROPE: Austria (**1**) *Hyphelia terrestris* var. *flava*, ad terram humidam in dumestis, non raro, Autumno, Oestrich, Fuckel. Fuckel Fungi Rhen. Exs. Supp. 1641 (Symb. Mycol.: 363 1869) (G, GRO, BM, FH) [DAOM 83327, MUCL 2258]. **Italy**: (**2**) *Botrytis epigaea* Link. Padova, ad terram udam umbrosam, December. 1874. Saccardo P.A. Mycotheca veneta [Cent. IV, 1876] 360. [MUCL 2283] (**3**) *Botrytis epigaea* Link, a) ad terram nudam in Horto Botanica Ticinensi, b) ad terram herbosam sub cupuliferis, ibidem. Ipse lege. Autumno. Forma b *rubella* ad var. *roseam* Sacc. ferendo mihi videtur, quamvis conidia paulum majora sint. Cavara F. Fungi Longobard. Exs. 145 (B, K-M, S, L 910.224-184) [DAOM 83335, MUCL 1805 = 2285]. (**3**) (**4**) *Botrytis epigaea* Link, ad terram, Florentiae, June 1891, N. Martely, Herb. Bresadola (S) [DAOM 83939, MUCL 2516]. **Germany** (**5**) *Hyphelia terrestris* ad vias nemorum, Grossen Garten, prope Dresden. Rabenh. Klotzschii Herb. Viv.

Mycol. I, 1846 (PR, PAV, BR) [DAOM 83895, MUCL 2432]. (**6**) *Hyphelia terrestris*, in parnecio Tyrois, Aug. 29 1859, P.A. Karsten (H) [DAOM 83902, MUCL 2469]. **Sweden** (7) *Hyphelia pulvinata* (Fr.) Juel, pä sandjord utmed en väg I baarskog, Uppland, Uppsala, Stadsparken nära Norby, Oct. 1933, Seth Lundell. Flora Suecica 1068 (UPS) [DAOM 83910, MUCL 2482]. (**8**) *Hyphelia pulvinata* (Fr.) Juel, pä naken jord I vågkant, Upplannd, Estuna, Ljushammaren, Malmö, Sept. 27 1955, Seth Lundell. Fungi Suecici (UP) [DAOM 83917, MUCL 2490]. (**9**) *sine nomine*, auf nackter Erde, in Park, Uppland, par. Solna, Aug. 1906, L. Romell, Herb. Mykol. Lars. Romell 17715 (S) [DAOM 83944, MUCL 2523]. (**10**) *Polyactis epigaea* (Link) Bon, ?*Hyphelia,* auf nackter Erde auf einen Pfaden Upl. Stockholm: Ekbacken, Jul. 22 1894, L. Romell. Herb. L Romell (S) [DAOM 83945, MUCL 2526]. **Netherlands** (**11**) *Ostracoderma*, op de gronde in loofbos, Ulvenhout, Ulvenhouter bos, Oct. 6 1959, R.A. Maasgesteranus 13025 (L) [DAOM 83936, MUCL 2514]. **France**: (**12**) *Hyphelia terrestris*, in umbrosis viis, Montmorency, St Germain, Fontainebleau Sept. 1842-1848, Roussel, Herb. E Roussel (PC) [DAOM 84661, MUCL 2703]. **Belgium** (**13**) *Chromelosporium*, sur le sol d'un chemin en forêt, Bois de Hevelee, Heverlee, Brab., G.L. Hennebert (MUCL 6701). (**14**) *Hyphelia*?, sur soil humide, au bord d'un chemin ombragé, Forêt de Soignes, Groenendael, Aug. 16 1960, G.L.H. (MUCL 1205, DAOM 74900).

NORTH AMERICA: CANADA: Ontario (**15**) *Ostracoderma*, Bells Corners, Ont. Sept. 10 1961, G.L. Hennebert (DAOM 83922, MUCL 2496). **USA: Virginia** (**16**) *Botrytis epigaea* Lk. var. *rosea* Sacc., on dead leaves, Limberlost, Shenandoah National Park, Va., Sept. 9 1937, J.A. Stevenson and V.K. Charles (BPI) [MUCL 2409]. (**17**) *Botrytis epigaea* Link, on soil under leaves, Arlington cementary, Virginia, May 22 1932, C.L. Shear (BPI) [DAOM 83870, MUCL 2427]. **Connecticut** (**18**) *Botrytis fulva* Link, near *B. epigaea,* on soil and grass, New Haven, Conn. July 6 1889, R. Thaxter, Herb. Atkinson (CUP) [DAOM 84696, MUCL 2879]. **Massachusetts** (**19**) *Botrytis spectabilis*, on rotten wood, Prospect Hill, Waltham, Oct. 1901, WG. Farlow (FH) [DAOM 84701, MUCL 2885]. (**20**) *Botrytis epigaea*, near *Hyphelia terrestris,* on rotten wood in humic litter, Bedford, Mass., Sept. 1901, W.G. Farlow (FH) [DAOM 84697, MUCL 2881]. **New Mexico** (**21**) *Rhinotrichum roseum*, on buried dead leaves, Chirlehunt, Sept. 1854. Herb. Currey (K-M) [MUCL 2300].

Conidia very verrucose (6–12 warts, median view)

EUROPE: **Sweden** (**2**) *Hyphelia terrestris*, on soil, Kronoparken, Uppsala, Uppland, Aug. 1916, H.O. Juel. Flora Suecica (UPS) [DAOM 83911, MUCL 2483]. (**3**) *Hyphelia pulvinata* (Fr.) Juel, pä naken jord I strandsnåret, Upland, Naturpark Bondkyrka , Värdsätra, Jul. 18 1930, Set Lundell. Flora Suecica 0798 (UPS) [DAOM 83909, MUCL 2481]. (**4**) *Hyphelia,* ad terram nudam sub *Corylus*, öland, Persmäs parish, Legenäs, Aug. 3 1953, J.A. Nannfeldt. Flora Suecica 13327 (UPS) [DAOM 83906, MUCL 2477]. **Denmark** (**5**) *Ostracoderma,* ad terrram in fageto, Sjealland, Hvaloo skov, Sept. 14 1952, Mykologisk Kongress, J.A. Nannfeldt. Flora Danica 12603 (UPS) [DAOM 83919, MUCL 2492]. **UK** (**6**) *Ostracoderma pulvinatum* Fr. Sibbertoft, 1873, Berkeley, Herb. Berk (K-M) (conidia with prominent blunt warts, drawn by Berk.) [MUCL 3497]. (7) *Phymatotrichum,* on burn ground and wood, Ashridge, Herts., England, Oct. 9 1955, D.A. Reid (K-M) [MUCL 2303]. **Germany** (**8**) *Botrytis epigaea* Link., ad terram humidam, non frequens. Aestate. circa Schlangenbad. L. Fuckel. Fuckel Fungi Rhen. Exs. 2301, 1871 (G, K-M, B, K, GRO) [MUCL 2282].

France (**9**) *Hyphelia terrestris*, ad vias in sylvis Fontainebleau, Aug. 4 1869, Roussel, Herb. E. Russel (PC) [DAOM 84662, MUCL 2704]. **Belgium** (**10**) *Chromelosporium*, sur le sol en forêt, Bois de Bonsecours, Blaton, Hainaut, G.L. Hennebert (MUCL 6332).

Conidia small, coarsely verrucose (4 –6 warts, median view)

EUROPE. Sweden (**1**) *Hyphelia*, on bare soil under stormfelled spruce, Gastrikland, Hills in Tolfforskogen, near Tolffors, Aug. 15 1954, J.A. Nannfeldt. Flora Suecica 11065a (UPS) [DAOM 83908, MUCL 2480].

NORTH AMERICA: Canada: Québec (**2**) *Botrytis*?, on humus, Morgan's woods, MacDonald College, near Montreal, Qué. Aug. 27 1941, R.F. Cain 12979 (DAOM 80136) [MUCL 2276]. **USA: New York** (**3**) *Phymatotrichum*, on soil, Lloyd Cornell Preserve, Slatterville, NY, Sept. 6 1952, W.W. Diehl (BPI) [DAOM 83889, MUCL 2426].

Re-evaluation of *Hyphelia terrestris*

Identification of the majority of the examined herbarium specimens found to be synnematous *Chromelosporium*-like species as *Hyphelia terrestris* is based only on the short macroscopical description and habitat information by Fries (1829): "effuse, strigoso-tomentosa, candida, medio evanescens, sporidiis subargillaceis ... in terra humosa, umbrosa, humida and denudata" and ignores Fries's synonymy.

The signed and dated specimens among the specimens examined show that this interpretation was accepted as early as in 1842 by Roussel, 1846 by Rabenhorst, 1861 by Fuckel, 1865 by Karsten, 1886 by Saccardo, 1901 by Farlow, 1920 by Juel, and by many afterwards. Taking into account Fries's protologue for *Hyphelia terrestris*, this traditional interpretation is actually a misapplication of the name.

Juel (1920) observed that the macroscopical description of *Hyphelia terrestris* in Fries (1829) matched well the collection he made near Uppsala in 1916 and a similar collection made in Mustiala, Finland in 1865 by P.A. Karsten, who identified it as *Hyphelia terrestris* (UPS). Convinced that the observed conidiogenesis in these specimens had to be that of *Hyphelia terrestris*, Juel emended the genus *Hyphelia* (for the part regarding *Hyphelia terrestris*) adding "rami conidiophori, apice non inflati, conidiis rotundis brevissime pedicellatis undique tecti" to the Fries description.

Hennebert (1973) accepted Juel's interpretation of *Hyphelia terrestris* as a *Chromelosporium*-like species and interpreted (erroneously) Juel's publication as a lectotypification of *Hyphelia terrestris*. Actually Juel did not typify the name from any authentic Friesian material or from the two specimens that he cited. Hennebert named the fungus *Chromelosporium*

tuberculatum (Pers.) Hennebert, *Trichoderma tuberculatum* being the first synonym cited by Fries but for which no type exists. As *Hyphelia terrestris* had been sanctioned (Fries 1829), the name was corrected to *Chromelosporium terrestre* (Fr.) M.B. Ellis.

Fries (1825) originally introduced the generic name *Hyphelia* for *Trichoderma roseum* Pers. [≡ *Trichothecium* Link, fide Hughes 1958]. Fries (1825) also added "Aliud genus, mucedineum, representat vulgatissimum *Trichoderma tuberculatum* Pers. cui accedunt multae species novae.... Sed de his plura in S. M. III" [a reference to the forthcoming Systema Mycologicum 3]. "Huic generi *Trichodermatis* nomen tribuerem, nisi *T. viridi* jam a Cel. Linkio affixum, quod sancte servandum videtur."

However, instead of creating his proposed new genus ('aliud genus'), Fries (1829) actually divided *Hyphelia* into two unranked infrageneric parts: *Thelephoroideae* (for *H. rosea*, *H. spadicea, H. fusca*, and *H. nigrescens*) and *Hyphomycetoidea* (for the new species *H. terrestris*, described only macroscopically and with four synonyms).

Fries (1849) reduced his concept of *Hyphelia* by omitting the type, *H. rosea* (Pers.) Fr. and chose section names (instead of adjectives) for the subdivisions: *Xylohypha* for *H. nigrescens* and *H. fusca*; and *Geohypha* for *H. terrestris.* The omission of the type, *H. rosea*, made *Hyphelia* Fr. 1849 an illegitimate later homonym of *Hyphelia* Fr. 1825, nom. sanct. The names of the subdivisions *Geohypha* Fr. and *Xylohypha* Fr. are legitimate and available for elevation to generic rank.

Fries (1829) described *Hyphelia terrestris* only macroscopically, with four synonyms: "*Trichoderma tuberculatum* Pers.; *T. nemorosum* Pers.; *T. laeve* Schum. (dubitans ipse citat *T. laeve* Pers.); and *T. varium* Ehrenb."

Searching for the type material of these names, I have been informed that no authentic specimen exists for *Hyphelia terrestris* in UPS or S, and no specimen of *Trichoderma tuberculatum* remains in L. Specimens of *Trichoderma laeve* and of *Trichoderma nemorosum* are preserved in L. and of *T. varium* in B and STR.

The examination of these authenticated specimens, one of *Trichoderma laeve*, (not the one labelled "*Trichoderma laeve*?" by Persoon), one of *T. nemorosum,* and three of *T. varium* revealed that they represent one species distinguished from *Chromelosporium*-like species by conidiogenesis.

Persoon (1796) describes *Trichoderma tuberculatum*, with ash grey conidia ("pulvere cinereo"), like the grayish conidia ("clair-cendré") of *T. nemorosum*, suggesting that *T. tuberculatum* was similar to *T. nemorosum*.

Persoon also described the conidia of *T. laeve* as yellow, as Schumacher described his *T. laeve*. Ehrenberg described conidia of *T. varium* as variable in colour, while Fries described them as "subargillaceis".

The other specimen, labelled '*Trichoderma laeve ?*' by Persoon, contains pieces of young conidiogenous cells of a *Chromelosporium*-like fungus, differing from his other labelled '*Trichoderma laeve,*' thus explaining Persoon's question mark. Persoon's doubt affected Fries (1829), who wrote "dubitans ipse citat *T. laeve* Pers." and cited instead "*T. laeve* Schum. ! Saell. 2 p. 236", a later homonym, of which Fries had seen a specimen. This illuminates the subsequent confusion between *Hyphelia terrestris* and the *Chromelosporium*-like fungi. This indicates also that the Friesian concept of *Hyphelia terrestris* differed from its traditional interpretation as a *Chromelosporium*-like fungus.

The most accurate interpretation of the nomenclature is to respect Fries's 1829 protologue of *Hyphelia terrestris* (a sanctioned name) and lectotypify the species by one synonym, of which material exists—*Trichoderma varium* Ehrenb.—rather than *T. tuberculatum* Pers. and *T. laeve* Schumach., which lack extant material.

With the generic name *Hyphelia* 1849 being illegitimate as a later homonym, *Hyphelia terrestris* therefore has status of type species of *Geophypha* Fr. which can be raised in rank to a genus. A parallel nomenclatural interpretation was made for *Hyphelia nigrescens* (Pers.) Fr., now regarded as the type species of the genus *Xylohypha* (Fr.) E.W. Mason by Deighton (1960).

Geohypha (Fr.) Hennebert, **stat. nov.**

MB 835628

≡ *Hyphelia* sect. *Geohypha* Fr., Summa Veg. Scand. 2: 447, 1849.

Type species: *Geohypha terrestris* (Fr.) Hennebert

= *Hyphelia* [unranked] *Hyphomycetoidea* Fr., Syst. Mycol. 3(1): 213, 1829, nom. sanct.

Ascomycota, ascomata unknown.

Hyphae septate, hyaline, intricate, irregular, branched.

Conidiophores short as lateral outgrowths from vegetatve hyphae bearing one conidium or long narrow sinuous and self-ramified branch, sparsely septate, bearing a conidium on each of the many lateral outgrowths and terminal ends.

Conidiogenesis thallic, solitary on hyphal outgrowths, secession schizolytic.

Conidia one-celled, globose or subglobose, sometimes napiform, hyaline, smooth to verrucose.

Habitat on soil.

Geohypha terrestris (Fr.) Hennebert, **comb. nov.** Figs 22, 23

MB 835629

≡ *Hyphelia terrestris* Fr., Syst. Mycol. 3(1): 213, 1829, nom. sanct.

≡ *Ostracoderma terrestre* (Fr.) Nannf., Fungi. Exsicc. Suec., Fasc. 53–54, Schedae: 40, 1959.

≡ *Chromelosporium terrestre* (Fr.) M.B. Ellis, More Demat. Hyphom.: 154, 1976.

= *Trichoderma tuberculatum* Pers., Ann. Bot. (Usteri) 15: 12, 1795 [as "*tuberculata*"].

≡ *Chromelosporium tuberculatum* (Pers.) Hennebert, Persoonia 7: 198, 1973.

= *Trichoderma laeve* Pers., Observ. Mycol. 1: 12, 1796.

= *Trichoderma varium* Ehrenb., Sylv. Mycol. Berol.: 22, 1803.

= *Trichoderma nemorosum* Pers.,Traité Champ. Comest.: 131, 1818.

= *Botrytis ceratioides* Peck, Annual Rep.New York State Mus. Nat. Hist. 35: 139, 1884.

= *Sporotrichum fossarum* Fautrey, Rev. Mycol. (Toulouse) 17: 71, 1893.

≡ *Ostracoderma fossarum* (Fautrey) S. Hughes, Canad. J. Bot. 36: 792, 1958.

Type: *Trichoderma ~~laeve Pers.?~~* [*laeve* Pers.? scratched off] *varium* m. [mei] ad Berol. in terra umbrosa humida, Thg. [Thiergarten] Berlin, 7/8 [Aug. 7] [scr. Erhenberg]. Herb. Ehrenberg (B, **MBT 392413, here designated as lectotype; isolectotypes:** [DAOM 83392, DAOM 83393, MUCL 2387, MUCL 2388]).

Colonies in small cushions, around 5 mm across, linked by a web-like mycelium, greyish yellow when fresh, brown when dried.

Hyphae narrow, irregular in diameter, 2–4 µm, sometimes inflated to 6 µm before septa and constricted at septa, hyaline, thin-walled, with abundant lateral narrower branchlets, fertile.

Conidiogenus cells either reduced to a conical to cylindrical lateral outgrowth of the hypha, 2–8 × 1–1.5 µm or (most often) developed into a long and narrow, sinuous, irregular, hypha, 8–50 × 1–1.5 µm, possibly ramified, producing ≤15–20 thallic conidia on lateral outgrowths and terminal ends.

Conidia thallic, solitary, borne on each hyphal outgrowth, seceding schizolytically through a 1–1.5 µm wide septum, one-celled, globose or subglobose, 4.5–6(–6.5) µm, hyaline, with a thick wall, at first smooth, soon becoming verrucose, with 12–15 warts in median view.

Habitat: on bare soil after rain in forest.

Comments—The type and authentic specimens of the species cited in the nomenclator are microscopically characterized as having narrow, sinuous conidiogenous hyphae and verrucose conidia mixed with young smooth conidia, the relative abundance depending on maturity of the fungus.

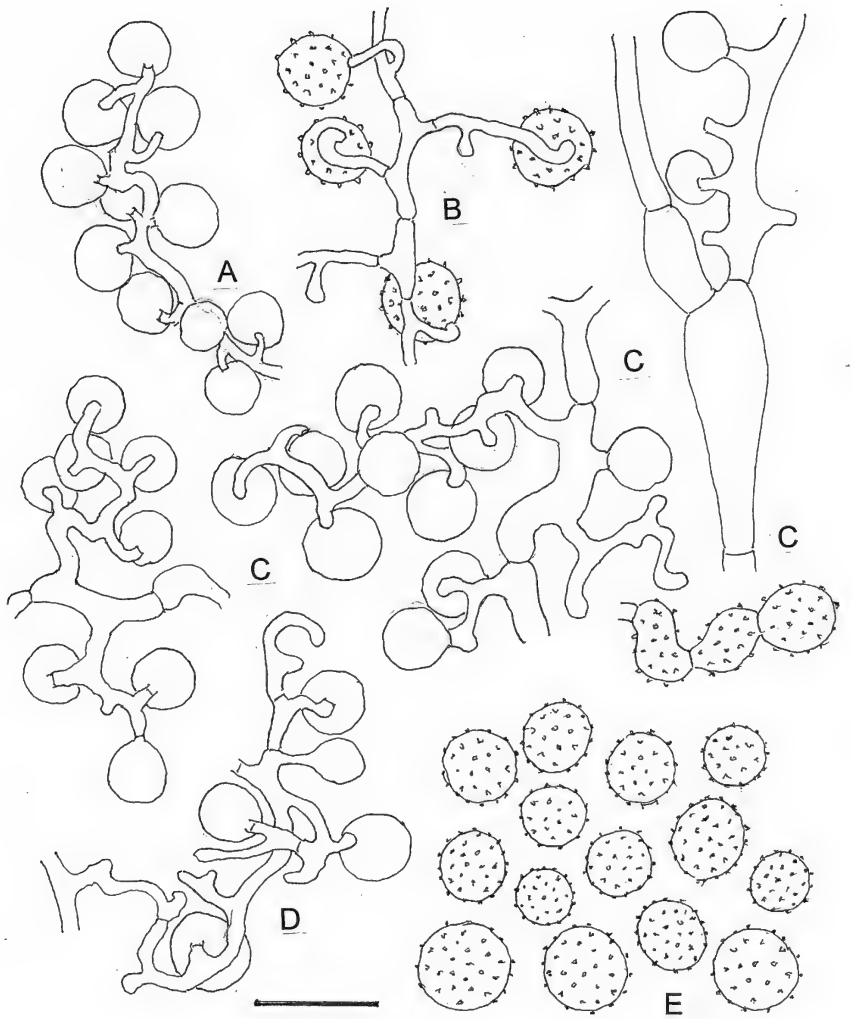

Fig. 22. ***Geohypha terrestris.*** A. *Trichoderma laeve* (MUCL 2462) conidiogenous cell with young conidia. B. *Trichoderma varium* (lectotype, MUCL 2387) with mature conidia. C. *Sporotrichum fossarum* (holotype, MUCL 2486b and isotype, MUCL 2394), conidia borne either solitary on hyphae, or numerous on tortuous conidiogenous hyphae. D. *Botrytis ceratioides* (holotype, MUCL 2467) conidiogenous cell, after release of some conidia. E. Mature conidia from these four specimens. Scale bar = 10 µm.

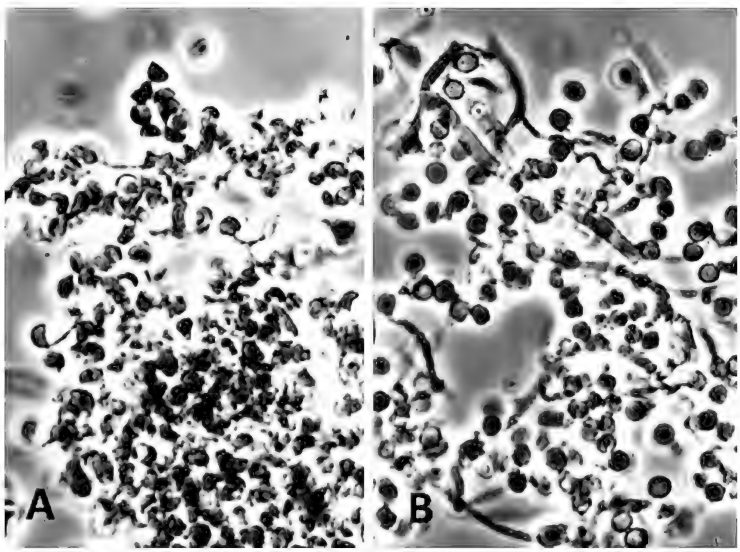

Fig. 23. A *Trichoderma laeve* Pers. (MUCL 2387). B. *Trichoderma varium* Ehrenb. (MUCL 2387) (Photos made by J.W. Carmichael, University of Alberta Mold Herbarium in 1961).

The size of the conidia obtained from each specimen drawn in Fig. 21 are *T. laeve* 4.5–5.5 (av. 5) µm, *T. varium* 4–6 (av. 5) µm, *B. ceratioides* 5–6(–6.8) (av. 5.7) µm, and *S. fossarum* 4–6.5 (av. 5) µm.

In 1961, I sent slides of *Trichoderma laeve* and *T. varium* from Persoon's herbarium to J.W. Carmichael for comparison with *Chrysosporium* species. He responded with photographs of the slides, and the opinion that the species were not congeneric with *Chrysosporium*.

The type of *Sporotrichum fossarum* contains two packets, one wrapped in paper contains soil in powder form on a piece of blotting paper (MUCL 2486a) and the other, in an aluminium sheet, contains the same soil kept compact and bearing the fungus (MUCL 2486b). The same fungus was distributed in G. Roumeguère's Fungi Selecti Exsiccati 6790 (MUCL 2394). The fungus has abundant vegetative hyphae that are often inflated before the septa and, when mature, verrucose conidia. Seeing the similarities with an *Ostracoderma* sp. sensu Juel, Hughes (1958) classified the species as *Ostracoderma fossarum*. Hennebert (1973) made it a synonym of *Chromelosporium tuberculatum,* an opinion later reported by Stalpers (1984). Revisiting the collection, I now assert that *Sporotrichum fossarum* is neither an *Ostracoderma* nor a *Chromelosporium* species but what is identified here as *Geohypha terrestris*.

Specimens examined

Type & authentic specimens: **EUROPE:** (**3**) *Trichoderma laeve* [scr. Persoon] Herb. Persoon (L 910.264.459) [MUCL 2462] (not L 910.264. 31, *Trichoderma laeve*? as written by Persoon). (**4**) *Trichoderma nemorosum* [scr. Persoon] Herb. Persoon (L 910.264-) [DAOM 83900, MUCL 2463]. (**5**) *Trichoderma varium* Ehbg. orig. [on soil] [scr. Ehrenberg] Herb. Schwaegrichen [stamped]" (STR) [DAOM 83898, MUCL 2441]. (**6**) "*Trichoderma varium* Ehrenb. [on soil, no locality, no date] [scr. Ehrenberg] (B) (DAOM 83394, MUCL 2389). **France** (**7**). *Sporotrichum fossarum* sp.n. Fautrey, sur la terre déjetée des fossés dans les bois humides. La Forêt de Clamecy. Eté 1894, Fautrey 212 (holotype) (UPS) [DAOM 83912, MUCL 2486-a, 2486-b]. (**8**) *Sporotrichum fossarum* n. sp. Fautrey, sur la terre rejetée des fossés dans les bois humides, été 1894. F. Fautrey (isotype). G. Roumeguère Fungi Selecti Exsiccati 6790 (Rev. Myc. 1895, p. 71, n.49) (G, NY) [MUCL 2394]. **Germany** (**9**) *Botrytis epigaea* Link, [var. *alba*] ad terram humidam, non raro, Aestate. Fuckel. Fungi Rhenani Fasc. II, 1863, n° 147 (BX 47111, GRO, S, K, FH) [MUCL 1798]. (Jahrb. Nassauischen Vereins. Naturk. 23: 363. 1870, p. 363 as, *Hyphelia terrestris* Fr. var. *alba,* nom. inval.). **USA: New york** (**10**) *Botrytis ceratioides* Peck, on decaying wood of *Tsuga canadensis,* Albany, June, leg. C.H. Peck. (holotype). See 35th Report p.139, 1884 (NYS) [MUCL 2467].

Other specimens: **EUROPE: Italy** (**11**) *Botrytis epigaea* Lk. f. *cinerea*, ad terram argillosam udam, Bosco Montello (Treviso), Sept. 1875. Saccardo Mycotheca Veneta 58 (K-M) [MUCL 2295]. **France** (**12**) *Trichoderma nemorosum* Pers. Autumno, ad terram, St Cloud, near Paris. Herb. de Candolle (G 005431) [DAOM 83992, MUCL 2392]. (**13**) *Trichoderma nemorosum* Pers. [on soil] Meudon, Augusto [no year] Herbier Léveillé in Herb. de Candolle (G 005432) [DAOM 83899, MUCL 2393]. **Sweden** (**14**) *Hyphelia*, on half buried birch branches in *Sphagnum* cushion, Gästrikland, NNW of Tolffors Swampy wood, Aug. 14 1950, J.A. Nannfeldt. Flora Suecica 11057 (UPS) [MUCL 2479a]. **Netherlands** (**15**) *Hyphelia terrestris* Fr. in terra argillacea, Lugd. Batava, 1844, Oudemans (GRO) [MUCL 2775]. **Denmark** (**16**) *Hyphelia terrestris* Fr. on bare soil, Saelland, Tisvilde Hegn., Oct. 4 1955, J.A. Nannfeldt. Flora Danica 14249 (UPS) [DAOM 84938a, MUCL 2495]. **Belgium** (**17**) *Trichoderma laeve* on bare soil, Lauzelle forest, Ottignies-LLN, Oct. 2017, G.L.H. (MUCL 56656).

NORTH AMERICA: USA: Nebraska (**18**) *Botrytis ceratioides* Peck [on leaves and mosses] Lincoln, 1200 ft, July 22 1890, T.A. Williams. Nebraska Flora 312 (BP) [MUCL 2423]. **Canada: Ontario** (**19**) *Rhinotrichum carneum* Ell. & Ev. on dead wood, Oct. 3 1896, Macoun, Ellis collection 185. (NY) [MUCL 2838]. (**20**) *Trichoderma laeve* Pers. on soil, Queen's University Biological Station, near Chafey's Lake, Ontario, July 12 1961, det. G.L. Hennebert (MUCL 2537).

Plicaria endocarpoides

Hennebert (1973) suggested that *Plicaria endocarpoides* could have a *Chromelosporium*-like asexual morph in culture based on a strain from J.W. Paden. The strain DAOMC 199565 produced on malt agar a conidial state having no similarity with *Chromelosporium* as illustrated here.

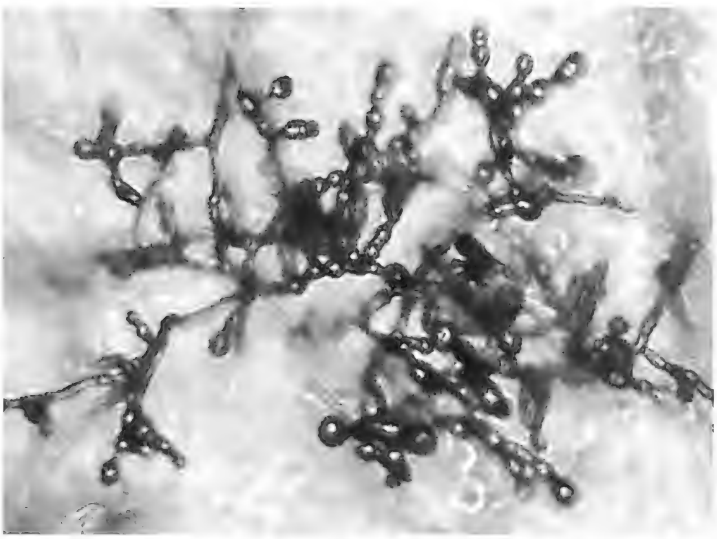

Fig. 24. *Plicaria endocarpoides* (DAOMC 199565 = MUCL 57202).
Conidial morph on malt-agar.

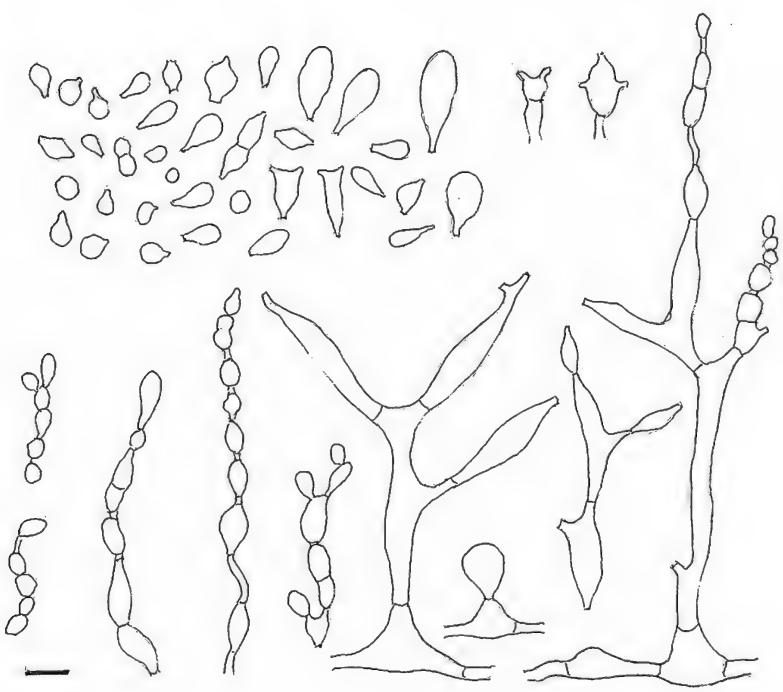

Fig. 25. *Plicaria endocarpoides* (DAOMC 199565 = MUCL 57202).
A. Conidiophores and conidia on malt agar. Scale bar =10 μm.

Plicaria endocarpoides (Berk.) Rifai, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 57(3): 255, 1968. FIGS 24, 25

≡ *Peziza endocarpoides* Berk., in Hooker, Fl. Nov.-Zel. 2: 199, 1855.

MYCELIUM hyaline prostrate on malt agar.

CONIDIOPHORES erect from creeping hyphae, one or pluri-celled, 10–110 µm high, the basal cell enlarged, triangular, the stipe often furcate.

CONIDIOGENUS CELLS intercalary or terminal of the conidiophore, cylindrical, fusiform, or triangular, 5–8 µm wide, producing acropetal solitary or branched chains of conidia, leaving unthickened scars at schizolytic secession.

CONIDIA hyaline, smooth, one-celled, some 2–3-celled, variable in shape, from globose (3–5 µm), to ovate, pyriform, or citriform (5–23 × 3–8 µm), most one celled, some septate, ramoconidia bearing one to three scars of attachment of simple or branched chains of conidia.

SPECIMENS EXAMINED: **CANADA: BRITISH COLUMBIA.** *Plicaria endocarpoides* (Berk.) Rifai, on burnt wood, slash and ash in coniferous forest, Lightning Lakes trail, Manning Provincial Park, British Columbia, June 9 1985, K.N. Egger 2044 in living culture (CCF 6892 = DAOMC 199565 = MUCL 57202).

Conclusion

In light of certain mononematous *Chromelosporium* asexual morphs actually representing *Peziza* or *Plicaria,* and certain synnematous *Chromelosporiopsis* asexual morphs representing *Pachyphlodes,* it was logical to emphasize a morphological character to distinguish the asexual morphs of these *Pezizaceae*. The chosen criterion of conidiophore fasciculation allows segregating similar conidial fungi not known to have sexual morphs into two genera—*Chromelosporium* around its mononematous type and the new genus *Chromelosporiopsis* for the synnematous species. *Chromelosporium* includes here five taxa with distinct morphologies. *Chromelosporiopsis* comprises so far two named taxa. In addition to the excluded taxa, there remain some doubtful taxa showing some (but insufficient) *Chromelosporiopsis* and a set of unidentified species detected among the herbarium specimens that further morphological investigation of might allow segregation into species. It is recommended that any new fresh conidial samples be not only genetically analysed but accurately described in their finest morphological details (including electron microscopy) to allow characterization and identification of these conidial fungi, many of which are likely to be asexual morphs of some known *Pezizaceae*.

Examination of many herbarium specimens revealed a long history of misapplication of the name *Hyphelia terrestris*, irrespective of Fries protologue of the species. A neotype of the species designated among the synonyms given by Fries allowed a new description of the species renamed *Geohypha terrestris*.

Acknowledgments

I am thankful to my colleagues, Dr. Keith Seifert (Adjunct Professor, Carleton University, Ottawa and formerly of Ottawa Research and Development Center, Agriculture and Agri-Food Canada) and Dr. David L. Hawksworth (Royal Botanic Gardens Kew), who thoroughly and accurately revised the manuscript in agreement with the present rules of nomenclature. I thank Dr Scott Redhead (Curator, National Mycological Herbarium (DAOM), Agriculture and Agri-Food Canada) for his appreciated nomenclatural advice and Dr Konstanze Bensch, (MycoBank curator, Westerdijk Fungal Biodiversity Institute, Utrecht) for the registration of names and types in MycoBank and her meticulous revision of all name citations. I thank Dr M. Scherrenberg and Dr J. Nuytinck of Naturalis Biodiversity Center in Leiden for the photographs and examination of the precious Persoon's material of *Isaria carnea* and Mr Jonathan Mack from Quebec for the photographs of *Chromelosporium coerulescens* I thank also Dr Shaun Pennycook and Dr Lorelei Norvell, editors of Mycotaxon, for editing the paper through very friendly exchanges. Also I remain grateful to the late Dr R.P. Korf of Cornell University, Ithaca, for his profound friendship and the multiple scientific exchanges and support for this research, particularly during 1973.

A personal note of gratitude to late Dr. Stanley J. Hughes

I am overall deeply grateful to late Dr. Stanley J. Hughes, a great mycologist, who was a great teacher and a loyal friend. After sending him some pages of my doctoral thesis on the genus *Botrytis*, inspired by some of his major publications, I received an invitation to apply for a National Research Council of Canada postdoctoral fellowship in the Mycology Section of the then Plant Research Institute of the Department of Agriculture Canada, in Ottawa, from October 1960.

My wife Lidwina and I arrived in Canada at the end of September 1960 and were received by Stanley and Lyndell Hughes. Once we were settled in the apartment they had carefully prepared for us, Stan introduced us to his colleagues of the Mycology Section in the Neatby Building at the Central Experimental Farm. The following day, Stan immediately began my training with a collecting trip to Gatineau Park near Ottawa (Fig. 26).

In the laboratory, showing great patience and efficiency with the student I then was (speaking only a few words of English accented French), Stan tested my skills with a simple project, describing *Balanium* from the DAOM herbarium. Then followed the description of *Arachnophora fagicola*, a new genus and species collected

in Belgium. As many as seven times, he asked me to improve the manuscripts I submitted to him, each word having to be useful and necessary.

To introduce me to ancient mycological literature, Stan asked me to clarify the complex history of *Oedemium didymum*. To describe the apothecial stage of certain *Botrytis* species reported in my doctoral thesis, Dr. J.W. Groves, in charge of the Mycology Section, with Dr. Elliot, introduced me to the in vitro production of the apothecia of *Botrytis* from living strains.

During the second year of the award, Stan (Fig. 27A) urged me to develop my research on the *Botrytis*-like fungi begun in my PhD thesis and which he classified in *Ostracoderma*. In 1973, incited by Dr Korf to publish, I revised some *Botrytis*-like genera and renamed *Ostracoderma* species in *Chromelosporium* (also described and illustrated in the present paper).

Those two years of mycological training with Dr. Hughes and Dr. Groves not only were essential to my career, but also provided the opportunity for creating an indestructible bond of respect and friendship. Later, Stan Hughes did to me the honour of visiting my laboratory at the UCL in Herverlee-Leuven in May 1973 (Fig. 27B) and in Louvain-la-Neuve in June 1994 (Fig. 27C) at the celebration of the Centenary of the MUCL fungus collection (Hennebert 2010). I remain very grateful to the late Stanley J. Hughes for the quality of my training and his friendship.

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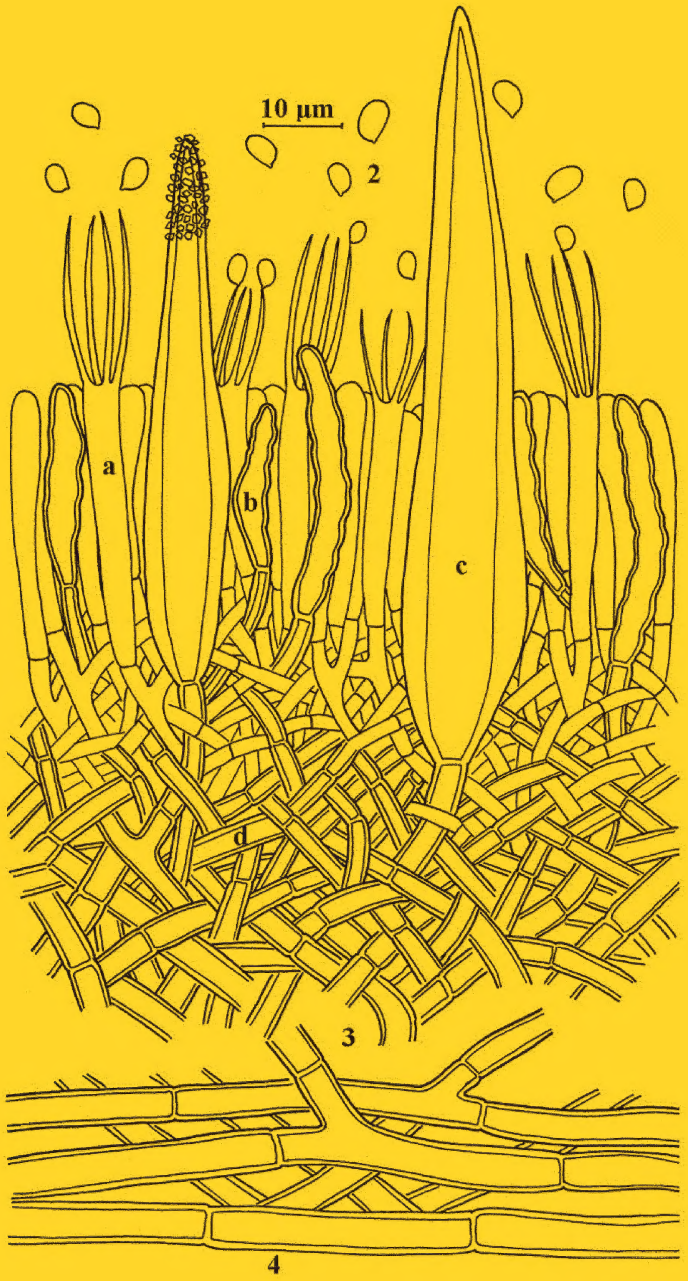

Hymenochaete longisterigmata sp. nov.
(Kaur, Singh, Dhingra,— Plate 1-1–4, p. 633)
Navpreet Kaur, *artist*